



Glyphosate and aminomethylphosphonic acid are not detectable in human milk^{1,2}

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ABSTRACT

Background: Although animal studies have shown that exposure to glyphosate (a commonly used herbicide) does not result in glyphosate bioaccumulation in tissues, to our knowledge there are no published data on whether it is detectable in human milk and therefore consumed by breastfed infants.

Objective: We sought to determine whether glyphosate and its metabolite aminomethylphosphonic acid (AMPA) could be detected in milk and urine produced by lactating women and, if so, to quantify typical consumption by breastfed infants.

Design: We collected milk (n = 41) and urine (n = 40) samples from healthy lactating women living in and around Moscow, Idaho and Pullman, Washington. Milk and urine samples were analyzed for glyphosate and AMPA with the use of highly sensitive liquid chromatography–tandem mass spectrometry methods validated for and optimized to each sample matrix.

Results: Our milk assay, which was sensitive down to 1 mg/L for both analytes, detected neither glyphosate nor AMPA in any milk sample. Mean \pm SD glyphosate and AMPA concentrations in urine were 0.28 \pm 0.38 and 0.30 \pm 0.33 mg/L, respectively. Because of the complex nature of milk matrixes, these samples required more dilution before analysis than did urine, thus decreasing the sensitivity of the assay in milk compared with urine. No difference was found in urine glyphosate and AMPA concentrations between subjects consuming organic compared with conventionally grown foods or between women living on or near a farm/ranch and those living in an urban or suburban nonfarming area.

Conclusions: Our data provide evidence that glyphosate and AMPA are not detectable in milk produced by women living in this region of the US Pacific Northwest. By extension, our results therefore suggest that dietary glyphosate exposure is not a health concern for breastfed infants. This study was registered at clinicaltrials.gov as NCT02670278. Am J Clin Nutr doi: 10.3945/ajcn.115.126854.

Keywords: AMPA, glyphosate, human milk, lactation, organic food, aminomethylphosphonic acid, breastfeeding, environmental contaminants, pesticide

INTRODUCTION

Glyphosate (N-phosphonomethyl glycine), a widely used herbicide patented as a phytotoxicant in 1974 (1), functions by

blocking the activity of 5-enolpyruvylshikimate-3-phosphate synthase (Enzyme Commission number 2.5.1.19), an enzyme required for the synthesis of tryptophan, phenylalanine, and tyrosine in plants and some microorganisms (2–5). Because these amino acids are not synthesized by humans, glyphosate would not be expected to have a physiological effect. Indeed, the human genome does not encode for 5-enolpyruvylshikimate-3-phosphate synthase, and a large body of epidemiologic and experimental literature supports the safety of glyphosate in mammals (5, 6). In addition, neither glyphosate nor its metabolite aminomethylphosphonic acid (AMPA)⁸ seem to bioaccumulate in animal tissues (7–9). In addition, most scientific evidence does not support contentions that glyphosate may cause cancer in humans, as recently concluded after a lengthy review by the European Food Standards Authority (10). The US Environmental Protection Agency (EPA) has authorized the use of glyphosate as an herbicide in noncrop and industrial areas since 1974 and in agriculture since 1976 (11). The safety of glyphosate use as an herbicide is periodically re-evaluated, with the last federal review completed in 1993 (12).

Despite its long-standing track record for safety, decades of research have resulted in a vast body of literature related to the clearance and disposition of ingested glyphosate. Studies in humans show that ~20% of diet-derived glyphosate is absorbed from the gastrointestinal tract, with the remaining ~80% excreted in the feces (13, 14), and studies conducted with rats suggest that nearly all absorbed glyphosate is rapidly excreted

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² Supplemental Figure 1 and Supplemental Table 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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⁸ Abbreviations used: AMPA, aminomethylphosphonic acid; EPA, US Environmental Protection Agency; LOD, limit of detection; LOQ, limit of quantification; RfD, reference dose.

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unchanged in urine (9, 15–18); little, if any, is metabolized to and excreted as AMPA. In fact, most AMPA in urine is thought to be the result of either the consumption of plants that have metabolized glyphosate into AMPA (12, 18, 19) and/or exposure to phosphonates found in detergents (20, 21).

Several studies have also investigated urine glyphosate concentrations of humans exposed to glyphosate via diet and other environmental sources (14, 22–25). These studies have consistently documented urine glyphosate concentrations of w 1–3 mg/L (in ppb), with the highest value being 233 mg/L (24). Curwin et al. (26) also reported urine glyphosate concentrations in 116 children living in “farm” and “nonfarm” households. Most samples (84%) had detectable concentrations with values similar to those reported in adults. There was no difference in urine glyphosate concentrations between children living in farm and nonfarm households (27). It is noteworthy that all measured urine glyphosate concentrations to date, even the highest, have not warranted a legitimate health concern based on the European Food Safety Authority’s allowable daily intakes and allowable operator exposure concentrations (14).

Of particular interest to our research group is the potential glyphosate exposure of infants during breastfeeding. Because there have been to our knowledge no studies published in peer-reviewed journals reporting glyphosate concentrations in human milk, this study (NCT02670278) was undertaken primarily to document typical glyphosate and AMPA concentrations in milk produced by lactating women living in the US Pacific Northwest—a highly productive agricultural region in which glyphosate-containing herbicides are routinely used (27). Maternal urine samples were also collected and analyzed. We hypothesized that concentrations of glyphosate and AMPA in milk and urine would be low, if even detectable. Important to testing this hypothesis was the use of newly optimized, matrix-specific assays with high sensitivities and specificities for the analytes (28).

METHODS

Human subjects

All procedures used in this study were approved by the Washington State University Institutional Review Board, and informed consent was obtained from each subject. A total of 41 healthy lactating women living in and around Pullman, Washington, and Moscow, Idaho, were included in the study, which was part of a larger investigation of international variation in human milk oligosaccharides and bacterial taxa as they relate to environmental exposure and sociocultural practices. To be eligible for participation, women had to be 1–3 mo postpartum, breastfeeding and/or pumping milk ≥ 5 times/d, and aged ≥ 18 y. Because we wanted to limit our subjects to healthy women who were nursing healthy infants, exclusion criteria included current breast infection, use of antibiotics in the previous 30 d, and having an infant with signs or symptoms of illness in the previous 7 d. Subjects completed a brief survey to document basic health and demographic variables, and body weight and height were measured at enrollment, which spanned from May 2014 through March 2015. All but 1 subject also completed a 5-question survey documenting potential glyphosate exposure from the environment and diet (Supplemental Figure 1).

Milk and urine collection and preservation

Milk and urine were collected between 0700 and 1100. After cleaning the breast (a step necessary to meet the needs of the larger, overarching project), w 30 mL milk was collected with the use of a Medela Symphony hospital-grade electric breast pump into a Medela Symphony single-use sterile collection container, immediately placed in ice, separated into aliquots while fresh, and then frozen at -20°C until analysis. A midstream urine sample was collected into a single-use sterile collection container. The sample container was immediately placed in ice, and urine was separated into aliquots and frozen at -20°C until analysis. One subject failed to provide a urine sample.

Glyphosate and AMPA analyses

Milk and urine samples were analyzed for glyphosate and AMPA at Monsanto with the use of liquid chromatography–tandem mass spectrometry methods optimized for and validated in each sample matrix (28). A Shimadzu Prominence 20A HPLC system coupled to an AB Sciex API 5500 triple-quadrupole mass spectrometer was used for analysis. Glyphosate and AMPA were quantitated with the use of multiple reaction monitoring. Two precursor-product ion transitions for each analyte and stable isotope labeled internal standard for each analyte were used to ensure the selectivity of the methods. Although 2 quantitative precursor-product ion transitions were monitored, the results were reported with the use of the most sensitive transition for each analyte. The assay was validated separately for milk and urine. Limits of detection (LODs) and quantification (LOQs) for glyphosate in milk were 1.0 and 10.0 mg/L, respectively; those for urine were 0.02 and 0.10 mg/L, respectively. LODs and LOQs for AMPA in milk were 1.0 and 10.0 mg/L, respectively; those for urine were 0.03 and 0.10 mg/L, respectively.

Glyphosate and AMPA concentrations in milk were independently confirmed by Covance with the use of the same liquid chromatography–tandem mass spectrometry method (28) with minor modifications, which included the use of an AB Sciex QTrap 5500 triple-quadrupole mass spectrometer. Because of differences in instrumentation, the LODs that used the more sensitive quantitative ion transitions were 6.0 and 9.0 mg/L for human milk glyphosate and AMPA, respectively, and the LOQ was 25.0 mg/L for both analytes.

It is noteworthy that duplicate aliquots (created from fresh milk at the time of collection) of each milk sample were sent directly, albeit separately, from Washington State University to Monsanto and Covance. Data generated by Covance were communicated directly to the principal investigators without prior disclosure to other coauthors.

Statistical analyses

All values for milk ($n = 41$) glyphosate and AMPA concentrations were below the LOD; thus, no statistical analyses on these data were warranted. For urine glyphosate and AMPA ($n = 40$), statistical analyses were conducted with the use of a generalized linear mixed model (SAS version 9.4; SAS Institute) assuming a Poisson distribution with a logarithmic link function. For concentrations less than the respective LOD values, one-half LOD (0.01 and 0.015 mg/L for glyphosate and AMPA, respectively) nominal values were used in the analyses. For

concentrations that fell between the LOD and LOQ, one-half LOQ (0.05 mg/L for both glyphosate and AMPA) nominal values were used in the analysis (29). All values presented represent means \pm 6 SDs.

RESULTS

Description of study population and glyphosate exposure

Basic demographic and anthropometric variables for the 41 study subjects are given in Table 1. Women were aged 29 \pm 5 y, 67 \pm 17 d postpartum, and had a BMI (kg/m²) of 26.8 \pm 8.6. Most (75%) lived in an urban or suburban nonfarming region of the Palouse (a geographical area encompassing southeastern Washington and northwestern Idaho), and most (58%) reported that they made no effort to eat foods characterized as organic, although they sometimes included them in their diets for convenience. Few subjects (15%) reported ever having personally mixed or used any type of weed killer; all but 1 of the women having reported ever doing so had mixed or used a weed killer containing glyphosate. In general, subjects were highly educated Caucasian women who participated in the study during either the summer or winter months.

Glyphosate and AMPA concentrations in milk

A summary of our findings concerning milk glyphosate and AMPA are found in Table 2. Regardless of whether the assays were conducted at Monsanto or Covance, none of the milk samples contained detectable amounts of either glyphosate or AMPA. As such, descriptive and statistical analyses were not warranted.

TABLE 1
Characteristics of the women participating in this study (n = 41)

Variable	Value
Age, y	29 \pm 5 ¹
Time postpartum, d	67 \pm 17
Parity, n	1.8 \pm 1.1
Body weight, kg	74.6 \pm 24.2
BMI, kg/m ²	26.8 \pm 8.6
Lived on or near a farm/ranch, ² %	25
Strictly or mainly organic food choices, ² %	42
Had at some time personally used or mixed any type of weed killer, ² %	15
Highest attained educational level, ² %	
High school	32
Undergraduate college degree	41
Graduate degree	27
Ethnicity, ² %	
Caucasian	93
African American	2
Latina	5
Season of sample collection, ² %	
Spring	12
Summer	46
Fall	17
Winter	27

¹Mean \pm 6 SD (all such values).

²Questionnaire data were missing for 1 woman; values represent those of the remaining 40 women.

TABLE 2

Mean glyphosate and AMPA concentrations (mg/L) in milk and urine produced by healthy women living in the US Pacific Northwest¹

Variable	Value
Milk (n = 41)	
Glyphosate ²	, LOD
AMPA ³	, LOD
Urine (n = 40)	
Glyphosate ⁴	0.28 \pm 0.38
AMPA ⁴	0.30 \pm 0.33

¹Values are means \pm 6 SDs. AMPA, aminomethylphosphonic acid; LOD, limit of detection; LOQ, limit of quantification.

²LOD = 1 and 6 mg/L when milk was analyzed at Monsanto and Covance, respectively; glyphosate could not be detected in any of the milk samples analyzed.

³LOD = 1 and 9 mg/L when milk was analyzed at Monsanto and Covance, respectively; AMPA could not be detected in any of the milk samples analyzed.

⁴Glyphosate and AMPA concentrations were less than the LOD or between the LOD and LOQ in 3 and 2 of the samples, respectively. For concentrations less than the LOD values, one-half LOD nominal values were used in the analyses; for those that fell between the LOD and LOQ, one-half LOQ nominal values were used. All analyses were conducted at Monsanto with an LOD and LOQ of 0.02 and 0.1 mg/L for glyphosate, respectively, and LOD and LOQ of 0.03 and 0.1 mg/L for AMPA, respectively.

Glyphosate and AMPA concentrations in urine

A summary of our findings concerning urine glyphosate and AMPA are found in Table 2 (raw data are available in Supplemental Table 1). Glyphosate was detectable in nearly all (n = 37) of the urine samples and was quantifiable in 29 of them. Glyphosate values ranged from below the LOD (, 0.02 mg/L) to 1.93 mg/L, with a mean of 0.28 \pm 0.38 mg/L. AMPA was also detectable in nearly all (n = 38) of the urine samples and quantifiable in 29 of them. Urine AMPA values ranged from below the LOD (, 0.03 mg/L) to 1.33 mg/L, with a mean of 0.30 \pm 0.33 mg/L. There were no significant effects of consuming organic compared with conventional foods or living on/near a farm compared with living in an urban/suburban region on concentrations of glyphosate in urine (P = 0.1870 and 0.8773, respectively) (Figure 1). Neither were there significant effects of consuming organic compared with conventional foods or living on/near a farm compared with living in an urban/suburban region on concentrations of AMPA in urine (P = 0.1414 and 0.2525, respectively) (Figure 2). Adjusting for potential covariates (age, time postpartum, BMI, parity) did not alter these conclusions. When raw, untransformed values were used in the analysis, there was a positive correlation (r = 0.57; P = 0.0001) between urinary glyphosate and AMPA concentrations. The strength of this association increased when log-transformed data were used (r = 0.68; P = 0.0001) (Figure 3).

DISCUSSION

The results herein provide evidence that the concentrations of glyphosate and AMPA in milk produced by healthy women are below the detection limits of available validated assays. In urine, glyphosate and AMPA were detectable in many samples, but concentrations were very low (, 0.02 to 1.93 and , 0.03 to 1.33 mg/L, respectively)—in fact, well below values reported in other

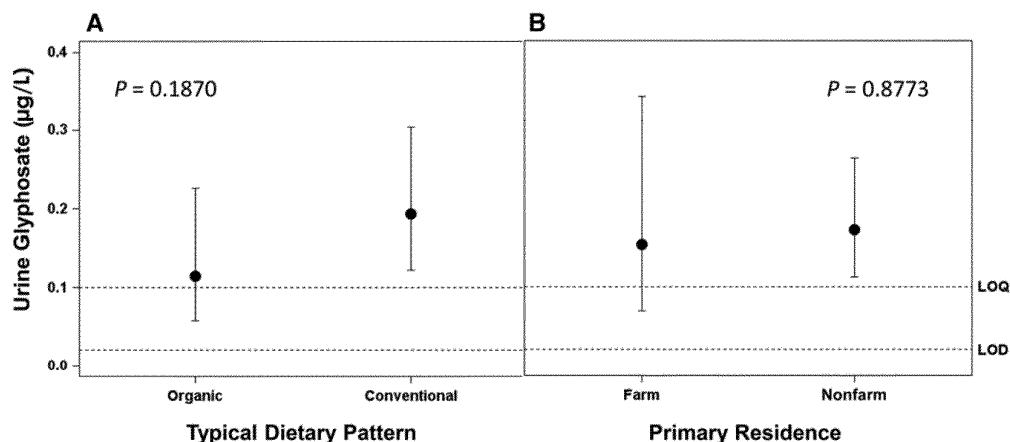


FIGURE 1 Estimated means and 95% CIs for urine glyphosate concentrations of typical self-reported dietary pattern types (A) ($n = 17$ and 23 organic and conventional, respectively) and primary residence types (B) ($n = 10$ and 30 on farm and nonfarm, respectively). Upper and lower reference lines (dashed) represent LOQ and LOD values, respectively. LOD, limit of detection; LOQ, limit of quantification.

healthy adult populations (, 0.15 to 29 and , 0.15 to 1.82 mg/L, respectively) (14, 24–26). To put these values in perspective, it is worth considering the EPA's reference dose (RfD) value for glyphosate. The RfD is an estimate of the quantity of a chemical that a person could be exposed to every day for the rest of his or her life with no appreciable risk of adverse health effects (30). The RfD for glyphosate is $1.75 \text{ g } \$ \text{ kg}^{-2.1} \$ \text{ d}^{-2.1}$; this value is based on a “no-effect” concentration in animals ($175 \text{ mg } \$ \text{ kg}^{-2.1} \$ \text{ d}^{-2.1}$) with a 100-fold safety factor (margin of exposure) (31). The EPA considers AMPA to be of similar or lesser toxicity than glyphosate and determined in 1994 that it should be exempt from regulation regardless of concentrations observed in food or feed (31). Thus, a 75-kg woman (typical weight for our study's participants) could consume as much as 131.25 mg glyphosate/d with no expected negative effects. If 20% of dietary glyphosate is absorbed (i.e., 20% bioavailability) (14) and 100% of absorbed glyphosate is excreted into urine, such an individual would be expected to excrete 26.25 mg/d (26,250 mg/d) glyphosate in her urine. In the current study, the highest reported

urine glyphosate concentration was 1.93 mg/L. As such, even allowing for a relatively high urine output (3 L/d), the highest glyphosate excretion in our study would be 5.79 mg/d, a value .4500 times lower than that which would be expected if the hypothetical mother described previously had consumed the RfD for glyphosate. The inclusion of AMPA, assuming equivalent toxicity, results in the highest excretion in our study of 2.58 mg/L (7.74 mg/d assuming 3 L urine/d) glyphosate + AMPA, an exposure .3000 times below the RfD; this combined calculation may become important if the EPA reconsiders the safety of AMPA (31).

Applying similar parameters and logic, a 5-kg infant can consume up to 8.5 mg/d (8500 mg/d) glyphosate and be below the RfD of this compound. Assuming a mean milk intake of 0.7 L/d (32–34) and a milk glyphosate concentration of 1 mg/L (the LOD value), then the maximum daily consumption of glyphosate by this hypothetical infant would be 0.7 mg/d—a value , 12,000 times that which is thought to signal any semblance of a health concern (31).

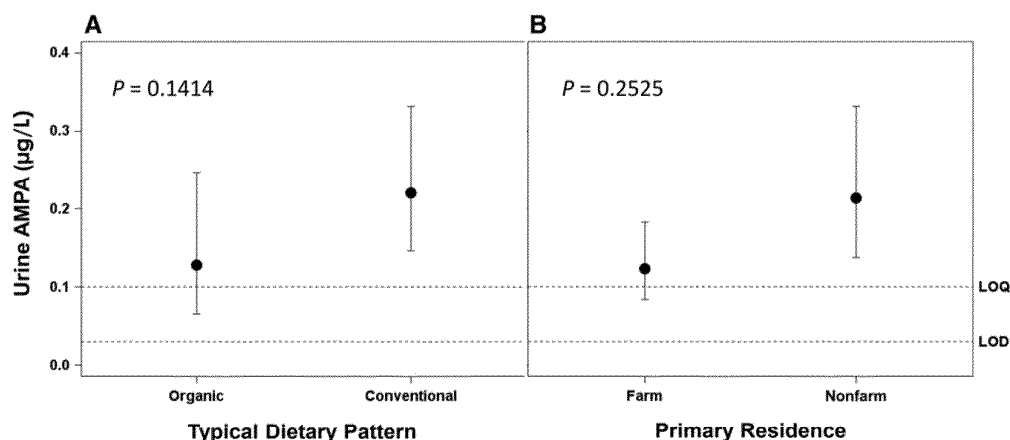


FIGURE 2 Estimated means and 95% CIs for urinary AMPA concentrations as they are related to typical self-reported dietary pattern types (A) ($n = 17$ and 23 organic and conventional, respectively) and primary residence types (B) ($n = 10$ and 30 on farm and nonfarm, respectively). Upper and lower reference lines (dashed) represent LOQ and LOD values, respectively. AMPA, aminomethylphosphonic acid; LOD, limit of detection; LOQ, limit of quantification.

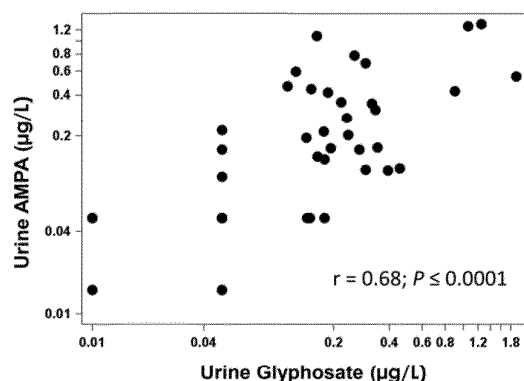


FIGURE 3 Correlation between urine glyphosate and AMPA concentrations. Vertical and horizontal axes represent the natural log scale ($n = 40$; 3 values hidden). AMPA, aminomethylphosphonic acid.

The observed correlation between urine glyphosate and AMPA concentrations is also of interest. Our reanalysis of previously published data from Hoppe et al. (20) suggests correlations (Pearson correlations of 0.40 and 0.68 for raw and log-transformed data, respectively) very similar to our data. Because the strength of association was greater with the use of the log-transformed data, it is likely that this relation is not proportional but rather nonlinear in nature. Whether the AMPA was derived from endogenous metabolism of glyphosate, consumed as a component of the diet, or resulted from exposure to AMPA-containing detergents, however, cannot be determined from our study.

There are some limitations that should be taken into consideration when interpreting the results of this study. First, our subjects were relatively homogeneous in terms of anthropometrics, demographics, and geographical place of residence. Future studies should consider recruiting women of varied educational and ethnic backgrounds living in different regions of the United States. Second, it is noteworthy that the larger international study from which these samples originate was not designed to detect small differences in urine glyphosate and AMPA concentrations based on dietary choices, location of residence (e.g., urban compared with rural), or occupational glyphosate exposure. Nevertheless, we thought it was of topical interest to preliminarily explore those hypotheses given the availability of information. We note, however, that detecting such small-effect sizes at statistically significant concentrations and adequate statistical power would require 4–5 times as many observations than used in this study. Subsequent research on this topic should consider increasing sample sizes to the largest extent possible while targeting enrollment of women who fit the hypotheses of interest, such as rarely or commonly consuming organic food, living on or off farms where glyphosate is used, and/or mixing and applying glyphosate as part of their livelihood. Studies might also consider investigating the possible effect of agricultural season on the outcomes of interest and the potential for breast infection (mastitis) to influence whether glyphosate and AMPA can be detected in a woman's milk. Investigators are also urged to collect urine samples from exclusively breastfed infants to verify the lack of glyphosate and AMPA exposure during this important period of the life cycle and consider collecting complete breast expressions in case glyphosate and AMPA concentrations change during feeding.

Last, studying potential glyphosate and AMPA exposures from other sources (e.g., environmental and supplementary foods) before and after weaning might be of interest. However, it is important to note that glyphosate exposure would need to be much higher than those reported herein for maternal or infant exposures to become a health concern.

The authors' responsibilities were as follows—MKM, MAM, DAG, and JLV: conceptualized and designed the study; MKM and DAG: designed the glyphosate exposure questionnaire; MKM: oversaw sample and data collection; JMC and KAL: collected the samples and administered the questionnaires; PKJ: oversaw the analysis of the samples at Monsanto; WJP and BS: carried out the statistical analysis; and all authors: read and approved the final manuscript. In 2014, MKM and MAM each received a \$10,000 unrestricted research gift from Monsanto; these funds were used to support their research related to human and bovine lactation. These funds were neither needed for nor used to cover the costs associated with the project described in this article, because the milk was already being collected for another project funded by the National Science Foundation (1344288) related to international variation in human milk composition and because additional expenditures associated with the collection of urine samples were negligible. All costs associated with the chemical analysis of milk and urine samples at both Monsanto and Covance were paid for directly by Monsanto. MKM and MAM were once reimbursed for costs associated with economy travel and basic accommodations incurred for a trip they made to St. Louis, Missouri, to discuss study design and assay development with coauthors DAG, PKJ, and JLV at Monsanto. DAG, PKJ, and JLV are employees of Monsanto, which manufactures glyphosate. None of the other authors reported a conflict of interest related to the study.

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Safety Evaluation and Risk Assessment of the Herbicide Roundup¹ and Its Active Ingredient, Glyphosate, for Humans

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Reviews on the safety of glyphosate and Roundup herbicide that have been conducted by several regulatory agencies and scientific institutions worldwide have concluded that there is no indication of any human health concern. Nevertheless, questions regarding their safety are periodically raised. This review was undertaken to produce a current and comprehensive safety evaluation and risk assessment for humans. It includes assessments of glyphosate, its major breakdown product [aminomethylphosphonic acid (AMPA)], its Roundup formulations, and the predominant surfactant [polyethoxylated tallow amine (POEA)] used in Roundup formulations worldwide. The studies evaluated in this review included those performed for regulatory purposes as well as published research reports. The oral absorption of glyphosate and AMPA is low, and both materials are eliminated essentially unmetabolized. Dermal penetration studies with Roundup showed very low absorption. Experimental evidence has shown that neither glyphosate nor AMPA bioaccumulates in any animal tissue. No significant toxicity occurred in acute, subchronic, and chronic studies. Direct ocular exposure to the concentrated Roundup formulation can result in transient irritation, while normal spray dilutions cause, at most, only minimal effects. The genotoxicity data for glyphosate and Roundup were assessed using a weight-of-evidence approach and standard evaluation criteria. There was no convincing evidence for direct DNA damage *in vitro* or *in vivo*, and it was concluded that Roundup and its components do not pose a risk for the production of heritable/somatic mutations in humans. Multiple lifetime feeding studies have failed to demonstrate any tumorigenic potential for glyphosate. Accordingly, it was concluded that glyphosate is noncarcinogenic. Glyphosate, AMPA, and POEA were not teratogenic or developmentally toxic. There were no effects on fertility or reproduc-

tive parameters in two multigeneration reproduction studies with glyphosate. Likewise there were no adverse effects in reproductive tissues from animals treated with glyphosate, AMPA, or POEA in chronic and/or subchronic studies. Results from standard studies with these materials also failed to show any effects indicative of endocrine modulation. Therefore, it is concluded that the use of Roundup herbicide does not result in adverse effects on development, reproduction, or endocrine systems in humans and other mammals. For purposes of risk assessment, no-observed-adverse-effect levels (NOAELs) were identified for all subchronic, chronic, developmental, and reproduction studies with glyphosate, AMPA, and POEA. Margins-of-exposure for chronic risk were calculated for each compound by dividing the lowest applicable NOAEL by worst-case estimates of chronic exposure. Acute risks were assessed by comparison of oral LD₅₀ values to estimated maximum acute human exposure. It was concluded that, under present and expected conditions of use, Roundup herbicide does not pose a health risk to humans. © 2000 Academic Press

Key Words: glyphosate; Roundup; herbicide; human exposure; risk assessment.

INTRODUCTION

History of Glyphosate and General Weed Control Properties

The herbicidal properties of glyphosate were discovered by Monsanto Company scientists in 1970. Glyphosate (Fig. 1) is a nonselective herbicide that inhibits plant growth through interference with the production of essential aromatic amino acids by inhibition of the enzyme enolpyruvylshikimate phosphate synthase, which is responsible for the biosynthesis of chorismate, an intermediate in phenylalanine, tyrosine, and tryptophan biosynthesis (Fig. 2). This pathway for biosynthesis of aromatic amino acids is not shared by members of the animal kingdom, making blockage of this pathway an effective inhibitor of amino acid biosynthesis exclusive to plants. Glyphosate expresses its herbi-

¹ Roundup is a registered trademark of Monsanto.

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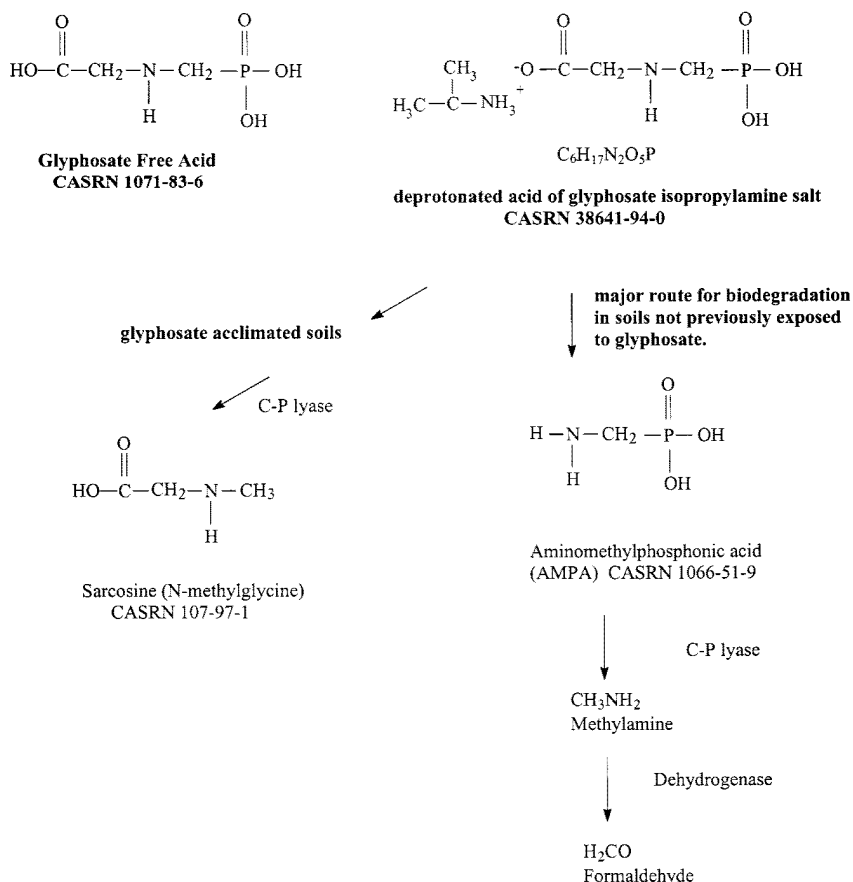


FIG. 1. A simplified pathway for degradation of glyphosate in the terrestrial environment. (Adapted from R. Wiersema, M. Burns, and D. Hershberger (Ellis *et al.*, 1999).)

cidal action most effectively through direct contact with foliage and subsequent translocation throughout the plant. Entry via the root system is negligible in terrestrial plants. For example, glyphosate applications will eliminate weeds around fruit trees in an orchard without harming the trees, provided that the leaves of the tree are not exposed. Glyphosate is predominantly degraded in the environment by microorganisms and through some limited metabolism in plants (Fig. 1); glyphosate ultimately breaks down to innocuous natural substances such as carbon dioxide and phosphonic acid.

Roundup herbicide, which contains glyphosate as the active ingredient, was first introduced in 1974 for nonselective weed control (Franz *et al.*, 1997). During the past 25 years of commercial use, growers, agricultural researchers, and commercial applicators, working in conjunction with Monsanto Company, have expanded the uses of Roundup. These uses have largely focused on inhibiting the growth of unwanted annual and perennial weeds, as well as woody brush and trees in agricultural, industrial, forestry, and residential weed control settings. Glyphosate-based products have been increasingly used by farmers in field preparation

prior to planting and in no-till soil conservation programs. The use of glyphosate in agriculture continues to expand particularly in applications involving plant varieties that are genetically modified to tolerate glyphosate treatment (Roundup-Ready³). Today, a variety of glyphosate-based formulations such as Roundup are registered in more than 100 countries and are available under different brand names. Although patents for this product held by Monsanto Company have expired in many countries, Monsanto continues to be the major commercial supplier of glyphosate and its formulations, worldwide.

Purpose and Scope

Glyphosate and Roundup herbicide have been extensively investigated for the potential to produce adverse health effects in humans. Government regulatory agencies in several countries, international organizations, and other scientific institutions and experts have reviewed the available scientific data and independently judged the safety of glyphosate and Roundup.

³ Roundup-Ready is a registered trademark of Monsanto.

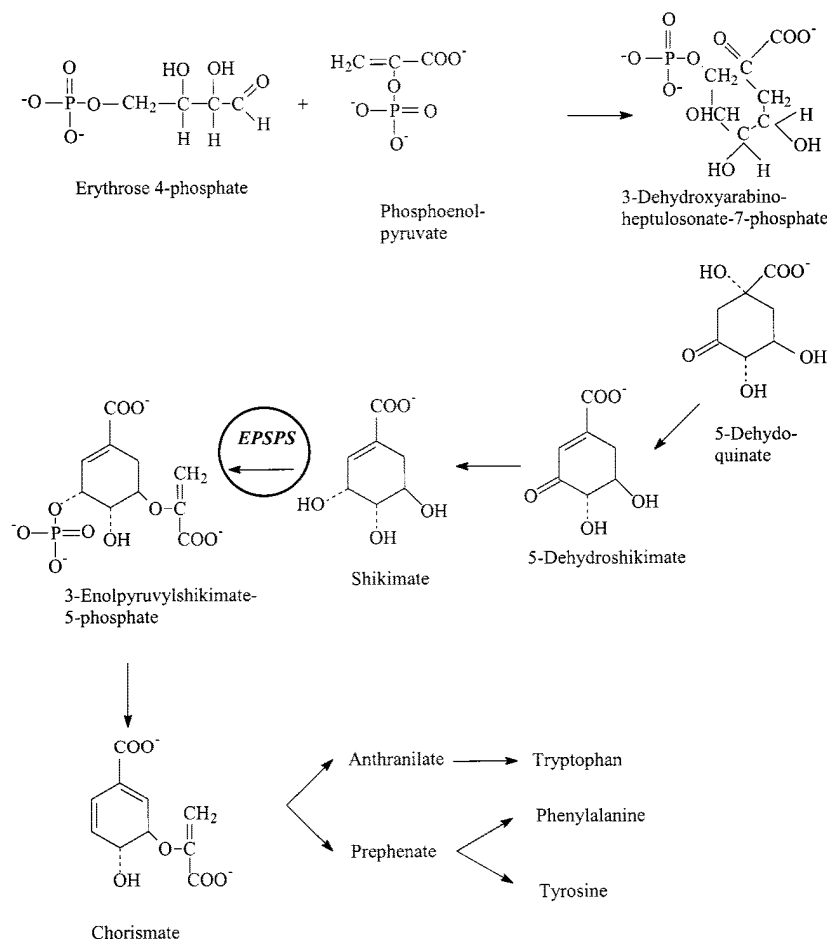


FIG. 2. Mechanism of action for glyphosate in plants. Glyphosate inhibits synthesis of essential aromatic amino acids by competitive inhibition of the enzyme enolpyruvylshikimate phosphate synthase (EPSPS).

Conclusions from three major health organizations [Health Canada, United States Environmental Protection Agency (U.S. EPA), and World Health Organization (WHO)] are publicly available (Health and Welfare Canada, 1986, 1992; U.S. EPA, 1993, 1997a, 1998a; WHO, 1994a). Those reviews, which have applied internationally accepted methods, principles, and procedures in toxicology, have discovered no grounds to suggest concern for human health. Data on Roundup and glyphosate are constantly reevaluated by regulatory agencies in a science-based process for many reasons including its volume of production and new uses. Nevertheless, questions regarding its safety are periodically raised.

The purpose of this review is to critically assess the current information pertaining to the safety of glyphosate and Roundup and to produce a comprehensive safety evaluation and risk assessment for humans. Certain sectors of the scientific and nonscientific communities have commented on the safety and benefits of pesticide use. With this in mind, parts of this assessment address specific concerns that have been raised

by special interest groups. This review will focus on technical glyphosate acid; its major breakdown product aminomethylphosphonic acid (AMPA);⁴ its Roundup formulations; and the polyethoxylated tallow amine surfactant (POEA), which is the predominant surfactant used in Roundup formulations worldwide. The review will evaluate data relating to toxicity based on exposure to Roundup and its components. The sources of information used in this review include studies conducted by Monsanto and published research reports dealing with glyphosate, AMPA, POEA and Roundup. The scientific studies conducted by Monsanto were per-

⁴ Abbreviations used: 8-OHdG, 8-hydroxyguanine; AMPA, aminomethylphosphonic acid; AUC, area under the curve; GLP, Good Laboratory Practices; IPA, isopropylamine; MCL, maximum contaminant level; MNPCE, micronucleated PCE; MOE, margin of exposure; MOS, margin of safety; MRL, maximum residue levels; NCEs, normochromatic erythrocytes; NOAEL, no-observed-adverse-effect levels; NOEC, no-observed-effect concentration; PCEs, polychromatic erythrocytes; POEA, polyethoxylated tallow amine; SCE, sister chromatid exchange assay; SSB, single-strand breaks; TMDI, theoretical maximum daily intake; UDS, unscheduled DNA synthesis.

formed for regulatory purposes and, thus, comply with accepted protocols and Good Laboratory Practices (GLP), according to standards of study conduct in effect at the time. Published research reports available in the general scientific literature range in quality from well-conducted investigations to those containing serious scientific deficiencies. Other sources of information, primarily reviews from regulatory agencies and international organizations, have also been used to develop this risk assessment. In this effort, the authors have had the cooperation of Monsanto Company that has provided complete access to its database of studies and other documentation. Glyphosate-based products are currently manufactured by a variety of companies worldwide. Some sources of information, including studies produced by manufacturers of glyphosate-based products other than Monsanto, are not generally available and as such were not considered for this risk assessment. Data for such products are proprietary and not readily available and therefore were not evaluated for inclusion in this risk assessment.

PRINCIPLES OF THE RISK ASSESSMENT PROCESS

The risk assessment process involves the characterization of toxicities and estimation of possible adverse outcomes from specific chemical exposures (CCME, 1996; Environment Canada, 1997; NRC, 1983; U.S. EPA, 1995, 1997a). The NRC (1983) and U.S. EPA Draft Cancer Risk Assessment Guidelines (1996) define risk characterization as the step in the risk assessment process that integrates hazard identification, dose-response assessment, and exposure assessment, using a combination of qualitative and quantitative information. Risk assessment can provide a comprehensive estimate of the potential effects in specific, well-defined, and described circumstances.

Hazard identification assesses the capacity of an environmental agent to cause adverse effects in experimental systems or humans. This is a qualitative description based on several factors such as availability of human data, data from laboratory animals, and any ancillary information (e.g., structure-activity analysis, genetic toxicity, pharmacokinetics) from other studies. Finally, a weight-of-evidence is prepared based on data accumulated from many sources, where a mode of action is suggested, responses in experimental animals are evaluated, and the relevance of these to human outcomes is discussed (U.S. EPA, 1995).

The determination of hazard is often dependent on whether a dose-response relationship is available (U.S. EPA, 1991). Hazard identification for developmental toxicity and other noncancer health effects is usually done in conjunction with an evaluation of dose-response relationships. The dose-response assessment evaluates what is known about the biological mode of action of a chemical and assesses the dose-response relationships on any ef-

fects observed in the laboratory. At this stage, the assessment examines quantitative relationships between exposure (or the dosage) and effects in the studies used to identify and define effects of concern.

The exposure assessment addresses the known principal paths, patterns, and magnitudes of human exposure and numbers of persons who may be exposed to the chemical in question. This step examines a wide range of exposure parameters including the scenarios involving human exposure in the natural environment. Monitoring studies of chemical concentrations in environmental media, food, and other materials offer key information for developing accurate measures of exposure. In addition, modeling of environmental fate and transport of contaminants as well as information on different activity patterns of different population subgroups can produce more realistic estimates for potential exposures. Values and input parameters used for exposure scenarios should be defensible and based on data. Any assumptions should be qualified as to source and general logic used in their development (e.g., program guidance, analogy, and professional judgment). The assessment should also address factors (e.g., concentration, body uptake, duration/frequency of exposure) most likely to account for the greatest uncertainty in the exposure estimate, due either to sensitivity or to lack of data.

A fundamental requirement for risk characterization for humans is the need to address variability. Populations are heterogeneous, so heterogeneity of response to similar exposures must also be considered. Assessments should discuss the dosage received by members of the target population, but should retain a link to the general population, since individual exposure, dosage, and risk can vary widely in a large population.

In addition to variability, uncertainty arises from a lack of knowledge about factors that drive the events responsible for adverse effects. Risk analysis is characterized by several categories of uncertainty including measurement uncertainty, uncertainties associated with modeled values, and uncertainties that arise from a simple lack of knowledge or data gaps. Measurement uncertainty refers to the usual error that accompanies scientific measurements as expected from statistical analysis of environmental sampling and monitoring. The assumptions of scientific models for dose-response or models of environmental fate and transport also have some uncertainty. Finally, in the absence of data, the risk assessor should include a statement of confidence that estimates or assumptions made in model development adequately fill the data gap.

Chemical Characterization and Technical Aspects of Roundup Formulations Addressed in This Review

Glyphosate is an amphoteric compound with several pK_a values. The high polarity of the glyphosate mole-

cule makes it practically insoluble in organic solvents. Glyphosate is formulated in Roundup as its isopropylamine (IPA) salt. Roundup is supplied as both dry and aqueous formulations at various concentrations; it is commonly formulated with water at 2.13 M (356 g/L free acid or 480 g/L IPA salt) with a surfactant added to aid in penetration of plant surfaces, thereby improving its effectiveness.

Technical-grade glyphosate acid manufactured by Monsanto Company averages 96% purity on a dry-weight basis. The remaining components are by-products of synthesis, whose individual concentrations are below 1%. This impurity profile has been identified and quantified during the development of the detailed manufacturing process. This information has been provided to and evaluated by a number of government authorities as part of the information supporting regulatory approval of Monsanto-produced glyphosate. All manufacturers of glyphosate-containing herbicides must meet similar regulatory requirements. This technical-grade glyphosate was used as the test material in the extensive toxicological testing discussed in this assessment. The identity of the impurities in technical-grade glyphosate has remained relatively unchanged over the course of the toxicological testing of the product described in the reports reviewed here. The findings of those studies, therefore, include any effects that could result from the impurities and are therefore embodied in the resulting hazard characterization and risk assessment.

Glyphosate acid is usually formulated with the organic base IPA to yield a more water-soluble salt. This salt, combined with water and a surfactant to improve performance in the field, comprise the principal glyphosate formulations sold worldwide under the Roundup family of brand names. The predominant surfactant used in Roundup products worldwide is a POEA, which is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids. This is the only surfactant considered in any detail in this review. Language considerations and differing business needs have resulted in the marketing of this formulation in some countries using a variety of other brand names (such as Sting, Alpheo, Azural, Faena, etc.). Roundup products are sometimes formulated with various amounts of surfactant, possibly containing additional surfactant components as substitutes for, or blends with, POEA. Most often, the concentration of glyphosate, on an acid basis, in these formulations is 360 g/L. This, however, is not always the case, and for certain markets where smaller quantities are needed, the base formulation is diluted with water to create more dilute products (e.g., 240, 160, 120, or 9 g/L).

For the purpose of this review, the term "Roundup" will be used to refer to this entire family of formulations, whose ingredients are qualitatively the same but

may vary in absolute amounts. In cases where these differences could lead to substantially different effects, these instances will be identified in the context of a comparison among different individual formulations and ingredients. Wherever possible, this document has converted measures to metric units of weight, volume, and area. Some reports of field studies have expressed concentrations in pounds, gallons, or acres, using units of acid equivalents or IPA salt active ingredient. The conversions have been made to simplify direct comparison of exposure and/or fate data whenever applicable.

Organization of Assessment

This assessment initially examines the metabolism and pharmacokinetic studies conducted with glyphosate and AMPA. This includes a review of studies conducted using oral and dermal routes of administration, as these are the predominant pathways of exposure to herbicides like Roundup. In the second section, the results of toxicology studies in animals are presented for glyphosate and AMPA followed by those conducted with Roundup and POEA. Consideration is then given to specific organ toxicity and other potential effects including endocrine disruption, neurotoxicity, and synergistic effects. In the next section, the effects of exposures to humans are discussed; both controlled studies and reports of occupational and other exposures are examined. This is followed by a detailed, worst-case exposure analysis for both children and adults. Finally, the results of the toxicological and exposure investigations are compared to provide an assessment of safety for humans. An outline of information presented in this assessment is shown below.

METABOLISM AND PHARMACOKINETICS GLYPHOSATE, AMPA, AND ROUNDUP

Glyphosate Oral Dosage Studies in Rats

Introduction

Three studies were conducted to investigate the pharmacokinetics of glyphosate following a single oral dose. In the first of two studies with Sprague-Dawley rats, glyphosate was administered at dose levels of 10 or 1000 mg/kg (Ridley and Mirley, 1988; Howe *et al.*, 1988). The second study was performed primarily to assess the distribution and nature of glyphosate-derived radioactivity in tissues following a 10 mg/kg dose (Brewster *et al.*, 1991). A third metabolism study was conducted by the National Toxicology Program (NTP) (1992) in the Fischer 344 strain of rat at dose levels of 5.6 and 56 mg/kg.

Two studies have been conducted to evaluate pharmacokinetic parameters in rats following repetitive oral exposure. In the first study, glyphosate was fed to Wistar rats at dietary concentrations of 1, 10, or 100

METABOLISM AND PHARMACOKINETICS GLYPHOSATE, AMPA, AND ROUNDUP Glyphosate Oral Dosage Studies in Rats Absorption Tissue Distribution Biotransformation/Excretion AMPA Single Oral Dose Study in Rats Glyphosate/AMPA Oral Studies in Non-rodents Glyphosate and ROUNDUP—Dermal Penetration	EVALUATION OF POTENTIAL SPECIFIC ORGAN/SYSTEM EFFECTS Salivary Gland Changes Potential for Endocrine Modulation Potential for Neurotoxicity Potential for Synergistic Interactions
TOXICOLOGY STUDIES WITH GLYPHOSATE AND AMPA Acute Toxicity and Irritation Studies Subchronic Toxicity Studies Chronic Toxicity/Oncogenicity Studies Reproduction/Developmental Toxicology Studies	HUMAN EXPERIENCE Irritation Studies Occupational Exposure Ingestion
TOXICOLOGY STUDIES WITH POEA AND ROUNDUP Acute Toxicity and Irritation Studies Subchronic Toxicity Studies Reproduction/Developmental Toxicology Studies	EXPOSURE ASSESSMENT Dietary exposure to Residues in Food Occupational Dermal and Inhalation Exposure During Application Non-occupational Exposure During Application Consumption of Water Reentry of Treated Areas Bystander Exposure During Application Possible Inadvertent Exposures Derived from Specific Activities Aggregate Exposure Estimates
GENETIC TOXICOLOGY STUDIES Review of Studies with Glyphosate, Formulations, and AMPA Evaluating Genotoxicity Data Weight-of-Evidence Narrative	RISK CHARACTERIZATION Identification of NOAELs Estimation of Risks to Humans from Acute or Chronic Exposure Overall Conclusion and Summary Statement

ppm for 14 days followed by a 10-day period during which there was no exposure to glyphosate (Colvin and Miller, 1973a). The second repetitive dosing study was conducted to determine if repeated administration alters the metabolic fate of glyphosate. In this study, pharmacokinetic parameters were evaluated in groups of Sprague±Dawley rats given glyphosate by oral gavage at a dose level of 10 mg/kg for either 1 or 15 consecutive days (Ridley and Mirley, 1988; Howe *et al.*, 1988).

Absorption

The absorption of orally administered glyphosate was shown to be incomplete. Following the administration of a single dose of glyphosate at 10 mg/kg, approximately 30 to 36% (males and females, respectively) of the dose was absorbed. This has been determined from measurements of the area under the curve (AUC) for whole blood (compared to the AUC for rats dosed intravenously) and the urinary excretion of radioactivity. These results were confirmed in the NTP study (1992), which showed that 30% of the administered 5.6 mg/kg dose was absorbed as determined by urinary excretion data. At the high dose of 1000 mg/kg, absorption appeared to be lower (approximately 19 to 23%) based on the percentage of material excreted in urine at 10 and 1000 mg/kg/day. In the 14-day repeated dose study conducted at dietary concentrations up to 100 ppm, it was estimated that 15% of the administered material was absorbed.

Tissue Distribution

The tissue distribution of glyphosate was investigated in Sprague±Dawley rats at 2, 6.3, 28, 96, and 168 h after the administration of a single 10 mg/kg oral dose (Brewster *et al.*, 1991). Tissue retention times were relatively short, and the vast majority of the body burden was unmetabolized parent glyphosate. Significant radioactivity (> 1% of administered dosage) was detected in the small intestine, colon, kidney, and bone.

Maximum concentrations in the small intestine (associated primarily with cells rather than contents) and blood were observed 2 h after oral glyphosate administration, while peak levels in other organs occurred 6.3 h after dosing. Levels of radiolabeled material in the small intestine, colon, and kidney declined rapidly. Radioactivity in bone steadily decreased over time, albeit at a slower rate than that observed in blood and other tissues. It was suggested that the slower elimination of glyphosate from bone may be due to reversible binding of the phosphonic acid moiety to calcium ions in the bone matrix; this type of binding has been shown to occur with glyphosate in soil (Sprankle *et al.*, 1975). Regardless of the mechanism involved, there has been no histological or hematological evidence of toxicity to bone in any of the toxicology studies conducted. Metabolite analysis showed that a minor metabolite was present in the gut content or colon tissue of a few animals. Analysis indicated that this metabolite was AMPA, but the small amount and transient nature of the material precluded further characterization. Essentially 100% of the radioactivity in all other

tissues/samples was shown to be parent glyphosate (Howe *et al.*, 1988).

When glyphosate was fed to Wistar rats in the diet for 14 days, steady-state tissue levels were reached within approximately 6 days of dosing (Colvin and Miller, 1973a). The highest glyphosate concentration was found in the kidneys (0.85 mg/kg tissue dry wt at the 100 ppm dosage level) followed in decreasing magnitude by spleen, fat, and liver. Tissue residues declined markedly after dosing was terminated. Ten days after dosing was discontinued, tissue levels ranged from only 0.067 to 0.12 mg/kg at the highest dosage tested. Data from the second multiple dosage study, in Sprague-Dawley rats, showed that repetitive dosing at 10 mg/kg body wt/day had no significant effect on the tissue distribution of glyphosate (Ridley and Mirly, 1988).

Biotransformation/Excretion

Orally administered glyphosate is poorly biotransformed in animals. It was shown to be rapidly excreted unchanged in the urine and feces of rats. For example, in the single dose study performed by NTP, it was reported that more than 90% of the radioactivity was eliminated in 72 h. The whole body elimination kinetics were evaluated for rats given the single 10 or 1000 mg/kg body wt and was found to be biphasic. The half-life of the a phase was approximately 6 h at both dose levels. The b phase half-lives ranged from 79 to 106 and 181 to 337 h for animals given the 10 or 1000 mg/kg doses, respectively. The feces was the major route of glyphosate elimination at all dose levels tested; approximately 62 to 69% of the administered dose was excreted in the feces. Less than 0.3% of an administered dose was recovered as CO₂ in expired air. In rats given glyphosate at 10 or 1000 mg/kg, the vast majority (97.5%) of the administered dose was excreted as unchanged parent material.

In the first multiple dosage study (1 to 100 mg/kg body wt/day for 14 days), urinary excretion accounted for less than 10% of the dosage, while 80 to 90% of the administered material was excreted in feces. The excreted material was shown to be essentially all unmetabolized glyphosate. Upon withdrawal of glyphosate, the amount in excreta dropped sharply, but plateaued temporarily after 4 days. This plateau was attributed to redistribution of mobilized tissue residues. Evaluation of the data from the second repeat dosage study conducted at 10 mg/kg body wt/day also showed that repetitive dosing (15 days) had no significant effect on the elimination of glyphosate as compared to single dosing.

AMPA Single Oral Dose Study in Rats

AMPA was administered via gavage at a dose of 6.7 mg/kg (Colvin *et al.*, 1973). Only 20% of the AMPA was

absorbed, while 74% of the administered dose was excreted in the feces over the 5-day period of experimental observation. The absorbed AMPA was not biotransformed and was excreted rapidly in the urine: approximately 65% of the absorbed dose was eliminated in the urine within 12 h, and essentially 100% was excreted between 24 and 120 h. Only trace residues (3 to 6 ppb) were detected in the liver, kidney, and skeletal muscle 5 days after dosing.

Glyphosate and AMPA Oral Studies in Nonrodents

Other studies have been conducted in which glyphosate or a glyphosate/AMPA mixture was administered to nonrodent species. Data from these investigations using rabbits, goats, and chickens have shown that the absorption, and resulting tissue levels, were low.

When a single oral dose of glyphosate (6 to 9 mg/kg) was administered to New Zealand white rabbits, more than 80% of the material appeared in the feces, indicating poor oral absorption (Colvin and Miller, 1973b). Tissue levels were less than 0.1 ppm by the fifth day after dosing.

Lactating goats were fed a diet containing 120 ppm of a 9:1 mixture of glyphosate and AMPA for 5 days (Bodden, 1988a). In a similar study, the same 9:1 glyphosate/AMPA mixture was fed to hens at dietary levels of 120 and 400 ppm for 7 days (Bodden, 1988b). The results from both studies indicated that 30% or less of the test material was absorbed. The concentrations of test material in goat milk ranged from 0.019 to 0.086 ppm at the end of the dosing period and declined to 0.006 ppm 5 days after the last dose.

When glyphosate was included in the diet of chickens at 120 ppm, residues in eggs obtained at the end of the dosing period ranged from 0.002 to 0.24 ppm and from 0.010 to 0.753 ppm at the 400 ppm dose level. When eggs were obtained 10 days after the last dose (120 ppm), residue levels ranged from nondetectable to 0.019 ppm.

Glyphosate and Roundup Dermal Penetration

The dermal penetration of glyphosate is very low based on results from studies in rhesus monkeys and *in vitro* studies with human skin samples. Maibach (1983) studied the *in vivo* dermal absorption of glyphosate when undiluted Roundup herbicide was applied to the skin of monkeys. Penetration was slow, as only 0.4 and 1.8% of the applied dose was absorbed over 24 h and 7 days, respectively. A second study in rhesus monkeys investigated the absorption of diluted glyphosate (1:29) to simulate a spray solution (Wester *et al.*, 1991). Dermal penetration was found to be 0.8 and 2.2% at low and high dose (500 or 5400 mg/cm², respectively). Wester *et al.* (1991) also reported that the *in vitro* percutaneous absorption of glyphosate through human skin was no more than 2% when applied for up

to 16 h either as concentrated Roundup or as a diluted spray solution. In another *in vitro* study, glyphosate absorption through human skin was measured during a 24-h exposure period and for up to 1 day afterward. When glyphosate was applied as formulated Roundup, a spray dilution of Roundup, or another concentrated glyphosate formulation (Franz, 1983), dermal penetration rates ranged from 0.028 to 0.152% for the three materials tested.

Summary

The pharmacokinetics of glyphosate and AMPA have been thoroughly evaluated in several studies. Both of these materials have phosphonic acid moieties with low pK_a s and therefore exist as charged molecules at the physiologic pHs found in the intestinal lumen. Only 15 to 36% of orally administered material given repeatedly, or as a single dose, was absorbed, thereby demonstrating that glyphosate and AMPA are poorly absorbed despite the prevailing acidic conditions. As expected for substances that are not well absorbed from the alimentary tract, the feces was the major route of elimination. The relatively small amounts of absorbed glyphosate and AMPA were rapidly excreted in urine almost exclusively as unchanged parent material. This was confirmed by the determination that levels of glyphosate and AMPA in peripheral tissues were low. Results from the multiple dose studies demonstrated that repeated oral dosing had no significant effect on elimination (compared to a single dose) and that glyphosate does not bioaccumulate. The dermal studies using glyphosate show low rates (less than 2%) of penetration with rhesus monkeys *in vivo* and human skin *in vitro*. Therefore, it is concluded that the potential for systemic exposure is limited by the combination of poor absorption and rapid excretion of glyphosate or AMPA after oral and/or dermal contact.

TOXICOLOGY STUDIES WITH GLYPHOSATE AND AMPA

Acute Toxicity and Irritation Studies

The acute toxicity of glyphosate and AMPA has been studied in laboratory animals. Oral and dermal LD_{50} values for glyphosate in rats are greater than 5000 mg/kg body wt (WHO, 1994a). The oral LD_{50} for AMPA in rats is 8300 mg/kg body wt (Birch, 1973). Using the acute toxicity classification system employed by the U.S. EPA, both glyphosate and AMPA are classified in the least toxic category (IV). These results show that the acute toxicity of glyphosate and AMPA is very low.

The potential for eye and skin irritation as well as dermal sensitization in response to glyphosate as the free acid has been evaluated in studies with rabbits and as the IPA salt in guinea pigs. In standard eye and skin irritation studies in rabbits, glyphosate (as the

free acid) was severely irritating to eyes but produced only mild skin irritation (WHO, 1994a). However, the IPA salt of glyphosate, which is the predominant form of glyphosate used in formulations worldwide, was nonirritating to rabbit eyes and skin (Branch, 1981). Glyphosate did not produce dermal sensitization in guinea pigs (Auletta, 1983a).

Subchronic Toxicity Studies

Glyphosate

Mouse studies. Glyphosate was administered to B6C3F1 mice in the diet at concentrations of 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm (NTP, 1992). Decreased body weight gain was observed at the two highest dietary levels in both males and females. At necropsy, the only significant finding was a dark salivary gland in one high-dose male. Alteration of parotid salivary glands was noted microscopically at and above the 6250 ppm dosage level. This histologic alteration consisted of microscopic basophilia of acinar cells and in more severely affected glands, cells, and acini appeared enlarged with an associated relative reduction in the number of ducts. The nature of this salivary gland change is further discussed in a later section. The sublingual and submandibular salivary glands were not affected. No treatment-related changes were observed in other organs, including the accessory sex organs.

There were several reasons to conclude that the salivary gland change observed is of doubtful toxicological significance. The complete discussion of the significance of changes observed in the salivary glands is presented in a later section (^a Evaluation of Potential Specific Organ/System Effects^o). Because these salivary gland changes are considered not to be relevant to humans, the no-observed-adverse-effect level (NOAEL) for glyphosate exposure in mice was based on the suppression of body weight gain and was set at 12,500 ppm (2490 mg/kg body wt/day, males and females combined).

In a separate study, glyphosate was fed to CD-1 mice for 13 weeks at dietary concentrations of 0, 5000, 10,000, or 50,000 ppm. The only treatment-related effect was decreased cumulative body weight gain in males and females (27 and 25% below controls, respectively) at the highest dosage tested (Tierney, 1979). When the submandibular salivary gland change was examined in this study, no changes similar to those described above for the parotid gland were observed. The NOAEL was 10,000 ppm (2310 mg/kg body wt/day).

Rat studies. Glyphosate was administered in the diet to F344 rats at levels of 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm for 13 weeks (NTP, 1992). The mean body weights of males were reduced in the 25,000

and 50,000 ppm groups (6 and 18%, respectively, below control); in females, there was only a marginal effect on body weight, as the mean weight of high-dose animals was approximately 5% below the control value. Small increases in one or more red blood cell parameters were reported in males at dosages of 12,500 ppm and above. Increased serum alkaline phosphatase and alanine aminotransferase values were noted at and above dietary levels of 6250 ppm (males) and 12,500 ppm (females). These increases were relatively small, not clearly related to dosage, and not associated with any histological changes of toxicological significance. At necropsy, no gross lesions related to glyphosate administration were observed. Other analyses in reproductive tissues are discussed in a later section. The parotid gland changes seen in B6C3F1 mice were also noted in the parotid and, to a lesser degree, submandibular glands of rats. The sublingual salivary gland was not affected at any dosage level. Salivary gland alteration was noted at the lowest dosage tested (209 mg/kg body wt/day for males and females combined), but for reasons described below, this effect can be ignored for purposes of evaluating safety in humans. The low dosage (3125 ppm or 209 mg/kg body wt/day), therefore, is considered to be a NOAEL based on changes in serum enzymes.

In another subchronic rat study, Sprague-Dawley rats were fed diets containing glyphosate at concentrations of 0, 1000, 5000, or 20,000 ppm for 90 days (Stout and Johnson, 1987). Submaxillary salivary glands were microscopically evaluated in this study and did not show the changes noted in the parotid and submandibular glands in the NTP study. No toxicologically significant effects were noted at any dosage level. Therefore, the NOAEL was set at the highest dietary exposure or 20,000 ppm (1445 mg/kg body wt/day, males and females combined).

Dog study. Glyphosate was administered by capsule to beagle dogs at dosages of 0, 20, 100, or 500 mg/kg body wt/day for 1 year (Reyna and Ruecker, 1985). There were no treatment-related effects in any of the parameters evaluated: clinical signs, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, gross pathology, and histopathology. Therefore, the NOAEL was 500 mg/kg body wt/day, the highest level tested.

Summary. Glyphosate has been evaluated in several subchronic toxicity studies in mice, rats, and dogs. The dosage levels used in these studies were very high, reaching dietary levels of 20,000 to 50,000 mg/kg body wt in rodent feeding studies and a dosage of 500 mg/kg body wt/day in a dog study. The primary finding was a decreased body weight gain in the rodent studies at the highest dietary concentrations tested (25,000 mg/kg body wt). This effect may have been due, at least in

part, to decreased food intake resulting from dilution of the caloric content of the diet (which contained 2.5 to 5% glyphosate) and/or reduced diet palatability. An alteration in the submandibular and/or parotid salivary glands (acinar cell hypertrophy and basophilic change) was observed in some of the rodent studies; the sublingual salivary gland was not affected in any study. For reasons discussed in a later section, this finding is not considered to be toxicologically significant or adverse. No salivary gland changes occurred in dogs. In summary, there were no treatment-related adverse effects in rats, mice, or dogs following glyphosate administration at extremely high levels for several weeks. Overall, it can be concluded that glyphosate when administered at daily dosages of up to 20,000 mg/kg body wt was well tolerated.

AMPA

Rat study. AMPA was administered in the diet to groups of Sprague-Dawley rats at dosage levels of 0, 400, 1200, or 4800 mg/kg body wt/day for 90 days (Estes, 1979). Changes that were noted included decreased serum glucose and elevated aspartate aminotransferase, but only at the highest dosage tested. An increase in calcium oxalate crystals was observed microscopically in the urine of high-dose animals, and urinary tract irritation was noted at the mid- and high-dose levels. Gross and microscopic pathology examinations did not reveal effects in any other organ. The NOAEL was 400 mg/kg body wt/day based on urinary tract irritation.

Dog study. AMPA was given to Beagle dogs via oral capsule at dosages of 0, 9, 26, 88, or 263 mg/kg body wt/day for 3 months (Tompkins, 1991). There was no treatment-related effect at any dosage level. Therefore, the NOAEL was 263 mg/kg body wt/day.

Summary. The subchronic toxicity of AMPA has been investigated in rats and dogs. Treatment-related effects were observed only at very high dosage levels. The NOAEL for rats was 400 mg/kg body wt/day, while no effects occurred in dogs even at the highest dosage tested (263 mg/kg body wt/day). Based on these results, it is concluded that the subchronic toxicity of AMPA, like that of parent glyphosate, is low.

Chronic Toxicity/Oncogenicity Studies

Glyphosate

Mouse study. CD-1 mice were readministered glyphosate in the diet at concentrations of 0, 1000, 5000, or 30,000 ppm for a period of 24 months (Knezevich, 1983). Total body weight gain in males was reduced at the end of the study (26% below control) at the highest dosage tested. Also in males, increased incidences of liver hypertrophy and necrosis were observed micro-

scopically at the high-dose level. An apparent increase in the occurrence of epithelial hyperplasia (slight-to-mild) of the urinary bladder in mid- and high-dose males was not considered treatment related because the incidence and severity of this lesion, common to the strain of animals used, showed no correlation with dosage. The NOAEL for chronic toxicity effects was 5000 ppm (885 mg/kg body wt/day) based on the effects on body weight and liver histology. In males, a small number of benign renal tubular adenomas were present in control and treated groups, but the incidences in treated groups were not significantly different by pairwise comparison to concurrent controls or by a trend test and were within the historical control range. Also, no related preneoplastic lesions were observed. Based on a weight-of-evidence evaluation, no treatment-related adenomas occurred. This conclusion was also reached by the U.S. EPA and an independent group of pathologists and biometricians under the auspices of U.S. EPA's Scientific Advisory Panel (SAP) (U.S. EPA, 1992a). The WHO (1994a) has also concluded that glyphosate did not produce an oncogenic response in this study. Accordingly, glyphosate is concluded to be noncarcinogenic in the mouse.

Rat studies. When glyphosate was fed to Sprague-Dawley rats at dietary concentrations of 0, 60, 200, or 600 ppm for 26 months, no treatment-related chronic effects were observed (Lankas, 1981). However, the incidence of interstitial cell tumors in the testes of high-dose males (6/50 or 12%) was above concurrent controls. This imbalance was not considered to be treatment-related because: (1) it was not accompanied by an increase in Leydig cell hyperplasia (an expected preneoplastic effect); (2) the incidence was within the historical control range; and (3) no increase was observed in the subsequent study conducted at higher dose levels (see below). Therefore, this study is concluded to reveal no oncogenic effect.

In a second study with the same strain of rat, glyphosate was administered at dietary concentrations of 0, 2000, 8000, or 20,000 ppm for two years (Stout and Ruecker, 1990). Treatment-related effects occurred only at the high-dose level and consisted of decreased body weight gain (23% below control at 20 months, the time of maximal depression) in females and degenerative ocular lens changes in males, as well as increased liver weights and elevated urine pH/specific gravity in males. There was a statistically significant increase in the incidence (9/60 or 15%) of inflammation in the gastric squamous mucosa of mid-dose females that was slightly outside of the historical control range (0 to 13.3%). Nevertheless, there was no dose-related trend across all groups of treated females, as inflammation was found in only 6 of 59 (10.2%) high-dose females. In males, there was no statistically significant increase in stomach inflammation in any group of treated animals,

and the frequency of this lesion fell within the historical control range. At the end of the study, usually a time when the occurrence of such lesions is greatest, there was a very low incidence of inflammation in treated animals examined. Considering all these factors, it is doubtful that the inflammation is treatment related. Small numbers of benign thyroid and pancreatic tumors were found in control and treated groups. The occurrence of thyroid and pancreatic tumors was judged to be sporadic and therefore unrelated to treatment for the following reasons: (1) the tumors observed were within the historical control range; (2) they did not occur in a dose-related manner; (3) they were not statistically significant in pairwise comparisons and/or trend tests; and (4) there were no increases in preneoplastic changes. Accordingly, glyphosate is concluded to be noncarcinogenic in the rat.

Based on these responses to prolonged exposure of glyphosate in rats, the 8000 ppm dosage level (409 mg/kg body wt/day, males and females combined) is concluded to be the NOAEL for chronic toxicity. This dosage was also determined to be the NOEL by the U.S. EPA (1993) and was considered to be the NOAEL by the WHO (1994a).

Summary. The chronic toxicity and oncogenic potential of glyphosate have been evaluated in one study with mice and two studies with rats. Few chronic effects occurred, and those were limited to the highest dietary levels tested (20,000 ppm in rats or 30,000 ppm in mice). Glyphosate was not oncogenic to either species. The studies and their results have been evaluated by a number of regulatory agencies and by international scientific organizations. Each of these groups has concluded that glyphosate is not carcinogenic. For example, the weight of evidence for carcinogenic hazard potential has been expressed by U.S. EPA using summary rankings for human and animal cancer studies. These summary rankings place the overall evidence in classification groups A through E, Group A being associated with the greatest probability of human carcinogenicity and Group E with evidence of noncarcinogenicity in humans. The U.S. EPA classified glyphosate in Category E, "Evidence of Non-carcinogenicity in Humans" (U.S. EPA, 1992a).

AMPA

Although lifetime studies were not conducted specifically with AMPA, its chronic toxicity and oncogenicity can be assessed by examining results from the second 2-year rat study with glyphosate (Stout and Ruecker, 1990). Analysis of the test material used in that study showed it contained 0.68% AMPA (Lorenz, 1994). On this basis, it can be concluded that AMPA was present at dietary levels of 13.6, 54.4, or 136 ppm at the 2000, 8000, or 20,000 ppm target concentrations for glyphosate, respectively. These dietary levels corresponded to

dosage levels of 0.69, 2.8, or 7.2 mg AMPA/kg/day. In that study, there were no chronic effects at the mid-dose level and no treatment-related tumors at any dosage tested. Therefore, it can be concluded that AMPA is not oncogenic at dosage levels up to 7.2 mg/kg body wt/day, and the NOAEL for chronic effects is at least 2.8 mg/kg body wt/day.

Reproduction and Developmental Toxicology Studies

Glyphosate

Reproductive toxicity. In the first of two multigeneration reproductive toxicity studies, glyphosate was administered to rats in the diet over three successive generations at dosage levels of 0, 3, 10, or 30 mg/kg body wt/day (Schroeder, 1981). An equivocal increase in unilateral renal tubule dilation was judged to be unrelated to treatment since a more extensive evaluation in the subsequent reproduction study conducted at much higher dose levels did not show such change. There were no treatment-related effects on mating, fertility, or reproductive parameters. The second study, also in rats, was conducted at dietary levels of 0, 2000, 10,000, or 30,000 ppm for two generations (Reyna, 1990). Decreased body weight gains were seen in parental animals at 30,000 ppm. Other effects at the high-dose level were reduced body weight gain in pups during the later part of lactation and an equivocal decrease in the average litter size. The NOAELs for systemic and reproductive toxicity were 10,000 ppm (≈ 694 mg/kg body wt/day) and 30,000 ppm (≈ 2132 mg/kg body wt/day), respectively.

In the subchronic toxicity study conducted in rats by NTP (1992), reduced epididymal sperm concentrations ($\approx 20\%$ below control) were reported in F344 rats at both the 25,000 and the 50,000 ppm levels. Nevertheless, all values were well within the normal range of sperm concentration values reported by the NTP in an analysis of their historical control data for these rodents (Morrissey *et al.*, 1988). As the apparent reductions were not related to dosage nor accompanied by decreases in epididymal weights or testicular sperm numbers/weight, the relationship to treatment is doubtful. Moreover, male fertility was not reduced in the reproduction study even at the highest dietary level tested (30,000 ppm).

An increase in estrous cycle length from 4.9 to 5.4 days was reported in the high-dose female F344 rats (50,000 ppm) (NTP, 1992). F344 rats, however, are known to exhibit highly variable estrous cycle lengths (4 to 6 days) leading Morrissey *et al.* (1988) to conclude that "stages of the estrous cycle are so variable [in F344 rats] that they may not be useful in assessing potential toxicity." Even if the estrous cycle length data were valid, they are of doubtful significance because the extremely high dosage associated with its occurrence. This dosage was several orders of magnitude

greater than any exposure ever likely to be experienced by humans (see Table 9 and discussion below). As no changes in sperm counts or estrous cycling were observed in mice treated at the same extremely high dosage levels, it is concluded that glyphosate does not adversely affect sperm concentration or estrous cyclicity at any relevant dosage.

Yousef *et al.* (1995) reported that subchronic glyphosate exposure produced effects on semen characteristics in New Zealand white rabbits; the effects included reduced ejaculate volume, sperm concentration, initial fructose levels, and semen osmolality. The study also reported evidence for increased abnormal and dead sperm. There were a number of serious deficiencies in the design, conduct, and reporting of this study which make the results uninterpretable. Only four rabbits per treatment group were used, suggesting questionable statistical validity for this study. The rabbits used in this study were small for their age (32 weeks at start of the treatment schedule, 50 weeks at termination of the experiment). Animals of similar age to those described in Yousef *et al.* (1995) are supplied by a number of commercial breeders. Normal adult New Zealand white rabbits 32 weeks of age (Harlan Sprague-Dawley, Indianapolis, IN) average 3.9 kg, with male rabbits occupying the lower portion of the weight range of 3.5 to 4.3 kg. Similar animals described by Yousef *et al.* (1995) had weights that were 0.5 to 0.9 kg ($16 \pm 25\%$) below historical norms. Weight deficiencies bring into question the health status and reproductive maturity of test animals used. Furthermore, the investigators did not actually quantify the two dosage levels used (referred to only as 1/10th and 1/100th of the LD_{50}), the purity of glyphosate, or the composition of the glyphosate formulation employed. Finally, Yousef *et al.* (1995) failed to state clearly the frequency of dosage applied to the animals in the protocol. With no accurate description of the method of delivery or quantity of chemical administered, a meaningful assessment of these studies cannot be made. Moreover a critical issue, especially in view of the authors' conclusions, is that the proper method of semen collection was not used, thereby invalidating any meaningful assessment of sperm viability, activity, and/or motility. Multiple ejaculates were not pooled to decrease the inter- and intra-animal variability in sperm number and concentration. Unfortunately, it was also unclear whether control animals were subjected to sham handling and dosing procedures, raising serious questions of indirect non-treatment-related effects given the known sensitivity of rabbits to stress. Additional points that seriously compromise this study include a lack of data for food consumption in control or treated animals, and failure to report variability in measurements for control and treated animals, preventing adequate statistical analysis to support conclusions of Yousef *et al.* (1995). Despite the 10-fold difference between the low- and high-

dose groups, dose-dependent responses were not observed. Sperm concentration data from both treated and control rabbits were well within the normal range of sperm concentration values previously reported for mature New Zealand rabbits (Desjardins *et al.*, 1968; Williams *et al.*, 1990). Based on these limitations as well as the other considerations, the data from this study cannot be used to support any meaningful conclusions.

Developmental toxicity studies. Glyphosate was administered by gavage to Sprague-Dawley rats at dosage levels of 0, 300, 1000, or 3500 mg/kg body wt/day on gestation days 6 to 19 (Tasker, 1980a). Severe maternal toxicity, including decreased weight gain and mortality (6 of 25 dams), occurred at the excessive dosage of 3500 mg/kg body wt/day and was accompanied by reduced fetal weights and viability and ossification of sternebrae. The NOAEL for maternal and developmental toxicity was 1000 mg/kg body wt/day.

Glyphosate was tested for developmental toxicity in rabbits following administration by oral gavage at dosage levels of 0, 75, 175, or 350 mg/kg body wt/day from gestation days 6 through 27 (Tasker, 1980b). Frequent diarrhea was noted in several high-dose animals. Deaths occurred in 1, 2, and 10 dams from the low-, mid-, and high-dose groups, respectively. Non-treatment-related causes of death (pneumonia, respiratory disease, enteritis, and gastroenteritis) were determined for the low-dose dam as well as 1 mid- and 3 high-dose animals. In the pilot teratology study conducted immediately prior to the definitive study, there was no mortality at dosages of 125 and 250 mg/kg body wt/day, while mortality occurred in 80% of the animals from the 500 mg/kg body wt/day group. When these pilot data are included in the overall analysis, and when mortality in the definitive study is redefined to eliminate non-treatment-related deaths, the overall mortality frequencies are 0, 0, 6, 0, 44, and 80% at 75, 125, 175, 250, 350, or 500 mg/kg body wt/day, respectively. This indicates an absence of a dose-response for treatment-related mortality below the 350 mg/kg body wt/day dosage. The death of the single mid-dose (175 mg/kg body wt/day) dam cannot be considered a treatment-related effect given the known vulnerability of rabbits to nonspecific stressors and the fact that no deaths occurred at a dosage of 250 mg/kg body wt/day in the pilot study. Therefore, the NOAEL for maternal toxicity must be represented by the 175 mg/kg body wt/day dosage, based on increased mortality and various clinical signs of toxicity at the next higher dosage tested. The 175 mg/kg body wt/day dosage level was also concluded to be the NOAEL by the WHO (1994a), while the U.S. EPA (1993) considers this level to be the NOEL. Although there were no effects in fetuses at any dosage level, the NOAEL for developmental toxicity was considered to be 175 mg/kg body wt/day due to the

insufficient number of litters available for examination in the 350 mg/kg body wt/day dosage group.

Summary. Results from several studies have established that glyphosate is not a reproductive or developmental toxicant. Glyphosate was evaluated in two multigeneration rat reproduction studies and in developmental toxicity studies in rats and rabbits. There were no effects on fertility or reproductive parameters, and glyphosate did not produce birth defects. Based on the lack of reproductive toxicity in two multigenerational studies conducted over a very wide range of dosages (; 3 to 2132 mg/kg body wt/day), there is no evidence of low-dose effects. The NOAELs for developmental toxicity are equal to or greater than the NOAELs for maternal effects, and the NOAEL for reproductive toxicity is greater than that for systemic toxicity. Therefore, there is no unique sensitivity from prenatal exposure (U.S. EPA, 1997a, 1998a). Apparent changes in sperm concentrations and estrous cycle length were reported in the NTP (1992) subchronic rat study at dosages of 1684 mg/kg body wt/day (sperm only) and 3393 mg/kg body wt/day (sperm and estrous cycle). Since these changes are not related to dosage, their magnitude falls well within the normal historical control range, and no such changes were observed in mice even at higher dosages, these findings are suspect and therefore difficult to assess. The reported findings in rats are considered biologically irrelevant because the dosages at which changes were reported are several orders of magnitude higher than any possible human exposure (see "Human Exposure"). The U.S. EPA has recently evaluated tolerance petitions under the Food Quality Protection Act of 1996 (FQPA) (Public Law 104-170) which includes special provisions to protect infants and children. The U.S. EPA concluded that there is "a reasonable certainty" that no harm will occur from aggregate exposure to glyphosate (U.S. EPA, 1997a, 1998a). The lowest NOAEL for any reproductive study is 175 mg/kg body wt/day in the rabbit developmental study.

AMPA

Reproduction and developmental toxicity studies. The potential for reproductive toxicity of AMPA can be assessed by examining the results from the two-generation rat reproduction study with glyphosate (Reyna, 1990). In this study, the glyphosate test material contained 0.61% AMPA (Lorenz, 1994), allowing calculation of dietary concentrations of AMPA at 0, 12.2, 61, or 183 ppm. Given that no effects were seen at the mid-dose level of this study, the overall NOAEL for AMPA is considered to be at least 61 ppm (; 4.2 mg/kg body wt/day, males and females combined) based on systemic (not reproductive) toxicity. In a developmental toxicity study, AMPA was administered by oral gavage to pregnant rats at dosage levels of 0, 150, 400, or 1000

TABLE 1
Acute Toxicity and Irritation of Roundup Herbicides and POEA Surfactant

Test material	Oral LD ₅₀ (mg/kg)	Dermal LD ₅₀ (mg/kg)	Inhalation (mg/L)	Eye irritation	Skin irritation
Roundup (41% IPAG) ^a	. 5000 (IV) ^b	. 5000 (IV)	3.18 (IV)	Severe (I)	Slight (IV)
POEA	1200	. 1260	Ø	Corrosive	Severe
Roundup T/O (18% IPAG)	. 5000 (IV)	. 5000 (IV)	. 5.7 (IV)	Moderate (III)	Essentially none (IV)
Roundup L & G Ready-to-Use (1% IPAG)	. 5000 (IV)	. 5000 (IV)	. 8.9 (IV)	Slight (IV)	Essentially none (IV)

^a IPAG, isopropylamine salt of glyphosate.

^b Roman numerals in parentheses denote EPA categories, where IV is the least toxic or irritating and I is the most toxic or irritating.

References. Roundup, oral and dermal LD₅₀ (WHO, 1994a); inhalation (Velasquez, 1983a); eye irritation (Blaszczak, 1990); skin irritation (Blaszczak, 1988). POEA, all studies (Birch, 1977). Roundup T/O, oral, dermal, eye, and skin (Auletta, 1985a±d); inhalation (Bechtel, 1987). Roundup L&G Ready-to-Use, oral, dermal, eye, and skin (Blaszczak, 1987a, b, c d, e); inhalation (Dudek, 1987).

mg/kg body wt/day on gestation days 6 through 15 (Holson, 1991). Slight decreases in maternal body weight gain and fetal body weights were noted at 1000 mg/kg body wt/day. Therefore, the NOAEL for maternal and developmental toxicity is 400 mg/kg body wt/day.

Summary. AMPA has been evaluated for potential adverse effects in reproductive and developmental studies with rats. In addition, the previously discussed reproductive tissues from the 3-month dog and rat toxicity studies with glyphosate, which contains AMPA (Estes, 1979; Tompkins, 1991), were examined for organ weight, macroscopic, and microscopic effects. No adverse effects have been observed in any of these evaluations. Therefore, it is concluded that the breakdown product, like the parent glyphosate, is not a reproductive or developmental toxicant.

TOXICOLOGY STUDIES WITH POEA AND ROUNDUP

Acute Toxicity and Irritation Studies

The acute toxicity of Roundup herbicide in rats, like that of glyphosate, is very low. The acute oral and dermal LD₅₀ values (Table 1) are greater than 5000 mg/kg body wt (WHO, 1994a). The 4-h inhalation LC₅₀ value in rats is 3.18 mg/L (Velasquez, 1983a). Based on these values, Roundup is placed in U.S. EPA's least toxic category (IV) for acute oral, dermal, and inhalation toxicity. Thus, the Roundup formulation is considered to be practically nontoxic by all these routes of exposure.

The acute toxicity of the surfactant, POEA, is somewhat higher than for Roundup formulation. Oral (rats) and dermal (rabbits) LD₅₀ values (Table 1) have been reported to be ; 1200 and . 1260 mg/kg, respectively (Birch, 1977). To put the acute toxicity in perspective, the oral LD₅₀ value for POEA in rats is similar to that

of vitamin A (1960 mg/kg) and greater than that of aspirin (200 mg/kg) (NIOSH, 1987). The oral LD₅₀ for POEA would place it in U.S. EPA's second-least-toxic category (III). Based on these considerations, POEA is considered to be only ^a slightly toxic and does not represent an acute toxicity hazard.

POEA was reported to be severely irritating to the skin and corrosive to the eyes when tested in rabbits (Birch, 1977). The irritation potential of POEA is consistent with the surface-active properties of surfactants in general. Surfactants with these properties are intentionally used in consumer products such as soaps, shampoos, laundry detergents, and various other cleaners. By virtue of their intended physicochemical properties, POEA and the other surfactants in consumer products interact with and solubilize lipid components characteristic of skin and mucous membranes.

Surfactants used in consumer products are effective at dilute concentration. POEA is not used in concentrated form but rather is formulated at lower concentrations into an end-use product (Roundup) and later diluted to very low levels, rendering it significantly less irritating. In standard studies with rabbits, concentrated Roundup herbicide was shown to be strongly irritating to eyes (Blaszczak, 1990) and only slightly irritating to skin (Blaszczak, 1988). When diluted to a concentration commonly used for most spraying applications (; 1%), Roundup was shown to be only minimally irritating to eyes and essentially nonirritating to skin (Table 1) (Blaszczak, 1987a,b). Standard dermal sensitization studies in guinea pigs were negative for both concentrated (Auletta, 1983b) and diluted (Blaszczak, 1987c) Roundup formulations. As will be discussed in a later section, controlled studies and other data from humans confirm that Roundup herbicide does not pose a significant eye or skin irritation hazard to humans.

Subchronic Toxicity Studies

POEA

Rat study. POEA was administered to Sprague±Dawley rats in the diet for 1 month at concentrations of 0, 800, 2000, or 5000 ppm (Ogrowsky, 1989). Body weight gains were reduced in males at the 2000 ppm level and in both sexes at the high-dose level. Prominent/enlarged lymphoid aggregates in the colon of high-dose females were associated with direct irritation/inflammatory effect of the test material. In a subsequent 3-month study with rats, POEA was administered in the diet at concentrations of 0, 500, 1500, and 4500 ppm (Stout, 1990). Among the animals from the high-dose group, effects noted included intestinal irritation, decreased food consumption and body weight gain, and some alterations in serum hematology/clinical chemistry parameters. Intestinal irritation was also observed in some animals from the 1500 ppm dosage level. Therefore, the NOAEL was 500 ppm in the diet (; 36 mg/kg body wt/day, males and females combined).

Dog study. The POEA surfactant was administered in gelatin capsules to beagle dogs for 14 weeks (Filmore, 1973). Because gastrointestinal intolerance (as evidenced by emesis and diarrhea) was observed at a preliminary stage, dosages were increased during the first 4 weeks of the study and then maintained at 0, 30, 60, or 90 mg/kg body wt/day for the final 10 weeks of the study. Body weights were reduced in high-dose animals; slight decreases in low- and mid-dose females were not always dose related and, thus, were of questionable significance. The biological significance of slight reductions in serum calcium and protein in mid- and/or high-dose dogs is also uncertain. While a definitive NOAEL was not established, the single significant finding in this study was the inability of dogs to tolerate surfactant ingestion on a daily basis due to gastrointestinal irritation.

Roundup

Sprague±Dawley rats were exposed to Roundup herbicide by inhalation using aerosol concentrations of 0.05, 0.16, or 0.36 mg/L for 6 h/day, 5 days/week for 1 month (22 total exposure days) (Velasquez, 1983b). The only change observed was evidence of respiratory tract irritation in high-dose females. This was considered to be a direct irritant response rather than a systemic effect. Therefore, the systemic no-observed-effect concentration (NOEC) was the highest dose or 0.36 mg/L. To put this value in perspective, the highest Roundup concentration measured in air during an applicator exposure study (Kramer, 1978) was 8.7×10^{-26} mg/L; this is approximately 40,000 times less than the NOEC from the inhalation study in rats.

The effect of dermal administration of Roundup to

rabbits was examined at dosage levels of 76 and 114 mg/kg body wt/day for 21 days (Killeen, 1975). Dermal irritation was observed at the application site, but there was no indication of systemic toxicity at either dosage tested.

A subchronic study with Brahman-cross heifers was carried out by administration of Roundup via nasogastric tube at dosages of 0, 400, 500, 630, or 790 mg/kg body wt/day for 7 days, after which animals were observed for an additional 14 or 15 days (Rowe, 1987). One cow died at the high-dose level, a death believed to result from gastric irritation and vomiting, followed by aspiration pneumonia. Diarrhea and body weight loss were observed at dosages of 630 and 790 mg/kg body wt/day, which was reduced to soft feces at the 500 mg/kg body wt/day dosage level. The NOAEL was 400 mg/kg body wt/day. It was estimated that the cows received dosages of Roundup herbicide on the order of 30 to 100 times greater than the dose typically applied to foliage for agricultural weed control purposes. Clearly, such exposures would never be achieved under normal agricultural use of glyphosate or Roundup. Thus, exposure to forage sprayed at recommended use should present no hazard to ruminant animals.

Summary

The subchronic toxicity of POEA has been assessed in 1- and 3-month studies with rats and in a 14-week study with dogs. Roundup herbicide has been evaluated for possible subchronic effects in an inhalation study with rats, a dermal study in rabbits, and an oral study with cattle. It was anticipated most observed effects would be related to the surface-active properties and associated irritation potential of surfactants. These studies confirm that irritation at the site of contact was the primary finding with the test material. In the oral studies with POEA and Roundup, some secondary effects were noted in addition to the gastrointestinal irritation. These included decreased food intake and body weight gain in rats and dogs and diarrhea and an associated slight body weight loss in cattle. There was no systemic toxicity in the inhalation and dermal studies with Roundup. No indication of specific target organ toxicity was observed in any of these studies. Therefore, it is concluded that the only changes produced were nonspecific effects that might normally be expected from repeated daily high-dose exposure to any material with significant surface-active properties.

Reproduction and Developmental Toxicology Studies

Developmental Study

POEA was administered by gavage to pregnant Sprague±Dawley rats on gestation days 6 through 15 at dosages of 0, 15, 100, and 300 mg/kg body wt/day

(Holson, 1990). Significant maternal toxicity was noted at the highest dosage tested, while minimal effects (decreased food consumption and mild clinical signs) occurred at the middose level. There were no effects in fetuses at any dosage. The NOAELs for maternal and developmental toxicity were shown to be 15 and 300 mg/kg body wt/day, respectively. The POEA surfactant is not a teratogen or a developmental toxin in rats.

Summary

The developmental toxicity of POEA has been evaluated in rats. Subchronic toxicity studies with the surfactant and/or Roundup herbicide have also been conducted in rats, rabbits, and dogs. In these studies, gross and microscopic pathology examinations were conducted on several reproductive tissues including ovaries, uterus, testes, and epididymis. No developmental effects or changes in reproductive tissues were found in any of these evaluations. There is no evidence that the surfactant or Roundup herbicide adversely impacts reproductive function.

GENETIC TOXICOLOGY STUDIES

Introduction

The consideration of the carcinogenic potential of Roundup, its active constituent ingredient glyphosate, or any of its other constituent ingredients can be assessed in a number of ways. Short-term tests for mutation, or for other evidence of genotoxic activity, allow identification of alterations in the genome. A primary purpose of such tests is to provide information on the production of heritable changes (mutations) that could lead to further adverse biological consequences. An initial and prominent question that tests for genotoxicity is designed to answer is whether the chemical (or any derivative) interacts directly with and mutates DNA (Williams, 1989). Such interactions are known to bring about changes in gene expression or to affect other key biological processes. However, there is clear evidence that some short-term tests demonstrate effects of toxicity that may or may not support direct interaction with DNA. Finally, some chemical exposures show no effect at low dosages and can be shown to be dependent on a threshold of exposure to produce an effect. The production of such indirect effects is often limited to conditions of high dose, which may be irrelevant to health risk assessment. The analysis that follows examines the most relevant endpoints to consider in evaluating evidence and any possible genotoxic action of Roundup in general and glyphosate in particular in terms of "a direct DNA effects" or "a indirect genotoxic effects". The database of results from tests related to effects on genetic material and the production of mutational events is presented in Table 2. The following discussion details individual results, where appropriate,

and then evaluates these results in a weight-of-evidence narrative that takes into account all the data available.

Glyphosate and Roundup

Glyphosate was negative in standard, validated mutagenicity assays conducted according to international guidelines and in GLP-compliant facilities. The database is, as is often the case, not entirely without some positive results, and these will be addressed below. Data related to endpoints for genotoxicity will be discussed in the following manner: First, *in vitro* and *in vivo* test results will be examined, followed by a discussion of evidence for production of DNA reactive species.

Gene Mutation Studies

Technical glyphosate has not been found to be mutagenic in several *in vitro* bacterial mutation assays using *Salmonella* and *Escherichia coli* tester strains. Multiple studies have been conducted in several strains of *Salmonella typhimurium* at concentrations up to and including cytotoxic levels with and without an exogenous source of metabolic activation (Li and Long, 1988; Moriya *et al.*, 1983; NTP, 1992; Wildeman and Nazar, 1982). In *E. coli*, glyphosate did not induce reversion at the *trp* locus in strain WP2 (Li and Long, 1988; Moriya *et al.*, 1983). These results confirm the absence of evidence in a sensitive system of mutation induction by glyphosate, even in the presence of various activating systems.

In mammalian cells, glyphosate was nonmutagenic at the HGPRT locus in Chinese hamster ovary cells treated *in vitro* with or without microsomal activation systems, even at doses that were toxic (Li and Long, 1988).

Several studies have tested herbicide formulations including Roundup, Rodeo, and Direct for mutation induction in bacteria. Four studies were negative (Kier *et al.*, 1997; Njagi and Gopalan, 1980), but one gave equivocal results (Rank *et al.*, 1993). The difference between herbicide formulations such as Roundup and glyphosate (usually as the IPA salt) used in genotoxicity assays is generally limited to the inclusion of a surfactant. Such surfactants include POEA and a similar, longer-chain tallow amine surfactant. Addition of surfactants generally increased the toxicity of the formulation compared to glyphosate alone in the *Salmonella* strains because these tester strains are particularly sensitive to substances that affect membrane surface tension. Toxicity of the formulations was observed at concentrations at which glyphosate content was only 0.5 mg/plate without S9 activation and 1.5 mg/plate when S9 was added. POEA is inactive in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and concentrations of up to 1.0 mg POEA/

TABLE 2

Summary of Results on the Genotoxicity of Glyphosate, Roundup, and Other Glyphosate Formulations

Test organism	Endpoint	Compound (purity)	Dose LED/ HID ^a	Evaluation ^b		
				Without S9	With S9	Reference
Gene mutation						
<i>S. typhimurium</i> TA98, TA100	Reverse mutation	Glyphosate (not speci@ed)	0.025 mg/plate	2	2	Wildeman and Nazar (1982)
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Glyphosate (not speci@ed)	5 mg/plate	2	S9 plant 2	Moriya <i>et al.</i> (1983)
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Glyphosate (98%)	5 mg/plate	2	2	Li and Long (1988)
<i>S. typhimurium</i> TA97, TA98, TA100, TA1535	Reverse mutation	Glyphosate (99%)	10 mg/plate	2	2	NTP (1992)
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538, TA1978	Reverse mutation	Roundup (glyphosate as isopropylamine salt, 36%)	5 mg/plate	2	2	Njagi and Gopalan (1980)
<i>S. typhimurium</i> TA98	Reverse mutation	Roundup (glyphosate 48%; POEA)	1.44 mg/plate	2	2	Rank <i>et al.</i> (1993)
<i>S. typhimurium</i> TA100	Reverse mutation	Roundup (glyphosate 48%; POEA)	0.72 mg/plate	2	1	Rank <i>et al.</i> (1993)
<i>S. typhimurium</i> TA98, TA100, A1535, TA1537	Reverse mutation	Roundup (glyphosate 30.4%; 15% POEA)	0.5 mg/plate	2	2	Kier <i>et al.</i> (1997)
<i>S. typhimurium</i> TA98, TA100, A1535, TA1537	Reverse mutation	Rodeo (glyphosate as isopropylamine salt, 54%)	5 mg/plate	2	2	Kier <i>et al.</i> (1997)
<i>S. typhimurium</i> TA98, TA100, A1535, TA1537	Reverse mutation	Direct (glyphosate as ammonium salt 72%; surfactant)	0.5 mg/plate	2	2	Kier <i>et al.</i> (1997)
<i>E. coli</i> WP2 <i>hcr</i>	Reverse mutation	Glyphosate (not speci@ed)	5 mg/plate	2	2	Moriya <i>et al.</i> (1983)
<i>E. coli</i> WP2 <i>hcr</i>	Reverse mutation	Glyphosate (98%)	5 mg/plate with S9, 1 mg/plate without S9	2	2	Li and Long (1988)
CHO cells (HGPRT)	Reverse mutation	Glyphosate (98%)	22.5 mg/mL	2	2	Li and Long (1988)
<i>D. melanogaster</i>	Sex-linked recessive lethals	Roundup (glyphosate 41%; POEA) (chronic to pupation)	1 mg/L (1 ppm)	1	0	Kale <i>et al.</i> (1995)
<i>D. melanogaster</i>	Sex-linked recessive lethals	Roundup (not speci@ed)		2	0	Gopalan and Njagi (1981)
Chromosomal aberration						
<i>Allium cepa</i> (onion root tip)	Chromosomal aberrations	Glyphosate (isopropylamine salt)	2.88 mg/L	2	0	Rank <i>et al.</i> (1993)
<i>Allium cepa</i> (onion root tip)	Chromosomal aberrations	Roundup (glyphosate 48%; POEA)	1.44 mg/L	1	0	Rank <i>et al.</i> (1993)

TABLE 2D Continued

Test organism	Endpoint	Compound (purity)	Dose LED/HID ^a	Evaluation ^b		
				Without S9	With S9	Reference
Peripheral lymphocytes (human) <i>in vitro</i>	Chromosomal aberrations	Glyphosate (. 98%)	0.56 mg/mL with S9, 0.33 mg/mL without S9	2	2	van de Waart (1995)
Peripheral lymphocytes (human) <i>in vitro</i>	Chromosomal aberrations	Glyphosate (. 98%)	1.4 mg/L	1	0	Lioi <i>et al.</i> (1998a)
Peripheral lymphocytes (bovine) <i>in vitro</i>	Chromosomal aberrations	Glyphosate (. 98%)	2.9 mg/L	1	0	Lioi <i>et al.</i> (1998b)
Rat bone marrow (<i>in vivo</i>) 6, 12, 24 h	Chromosomal aberration	Glyphosate (98%)	1.0 g/kg	2	0	Li and Long (1988)
Peripheral blood (human) <i>in vitro</i>	SCE	Roundup (not speci@ed)	2.5 mg/mL	6	0	Vigfusson and Vyse (1980)
Peripheral blood (human) <i>in vitro</i>	SCE	Glyphosate (99.9%)	1.0 mg/mL	1	0	Bolognesi <i>et al.</i> (1997)
Peripheral blood (human) <i>in vitro</i>	SCE	Roundup (glyphosate 30.4%; 15% surfactant)	0.1 mg/mL	1	0	Bolognesi <i>et al.</i> (1997)
Peripheral blood (human) <i>in vitro</i>	SCE	Glyphosate (. 98%)	1.4 mg/L	6	0	Lioi <i>et al.</i> (1998a)
Peripheral lymphocytes (bovine) <i>in vitro</i>	SCE	Glyphosate (. 98%)	2.9 mg/L	6	0	Lioi <i>et al.</i> (1998b)
<i>V. faba</i> (root tips)	Micronucleus test	Solado (glyphosate 21%)	1.4 mg/g soil	2	0	De Marco <i>et al.</i> (1992)
Mouse bone marrow (<i>in vivo</i>), dietary for 13 weeks	Micronucleus test	Glyphosate (99%)	11,379 mg/kg/day	2	0	NTP (1992)
Mouse bone marrow (<i>in vivo</i>) ip injection, 24 h, 48 h	Micronucleus test	Glyphosate (not speci@ed)	200 mg/kg	2	0	Rank <i>et al.</i> (1993)
Mouse bone marrow (<i>in vivo</i>) ip injection, 24 h	Micronucleus test	Roundup (glyphosate 48%; POEA)	200 mg/kg	2	0	Rank <i>et al.</i> (1993)
Mouse bone marrow (<i>in vivo</i>) ip injection	Micronucleus test	Glyphosate (99.9%)	300 mg/kg	1	0	Bolognesi <i>et al.</i> (1997)
Mouse bone marrow (<i>in vivo</i>) ip injection	Micronucleus test	Roundup (glyphosate 30.4%; 15% surfactant)	135 mg/kg	1	0	Bolognesi <i>et al.</i> (1997)
Mouse bone marrow (<i>in vivo</i>) ip injection	Micronucleus test	Roundup (glyphosate 30.4%; 15% POEA)	555 mg/kg	2	0	Kier <i>et al.</i> (1997)
Mouse bone marrow (<i>in vivo</i>) ip injection	Micronucleus test	Rodeo (glyphosate IPA 54%; water)	3400 mg/kg	2	0	Kier <i>et al.</i> (1997)
Mouse bone marrow (<i>in vivo</i>) ip injection	Micronucleus test	Direct (glyphosate 72% as NH ₄ salt; surfactant)	365 mg/kg	2	0	Kier <i>et al.</i> (1997)
Mouse (<i>in vivo</i>) gavage	Dominant lethal	Glyphosate (98.7%)	2000 mg/kg	2	0	Wrenn (1980)
DNA damage/reactivity						
<i>B. subtilis</i> H17, rec1; M45, rec2	rec-assay	Glyphosate (98%)	2 mg/disk	2	2	Li and Long (1988)
Rat hepatocytes (exposed <i>in vitro</i>)	UDS	Glyphosate (98%)	0.125 mg/mL	2	2	Li and Long (1988)

TABLE 2D Continued

Test organism	Endpoint	Compound (purity)	Dose LED/HID ^a	Evaluation ^b		
				Without S9	With S9	Reference
Mouse ip exposure (<i>in vivo</i>)	DNA adducts	Glyphosate (isopropylamine salt)	270 mg/kg	2	0	Peluso <i>et al.</i> (1998)
Mouse ip exposure (<i>in vivo</i>)	DNA adducts	Roundup (30.4% glyphosate isopropylamine salt; 15% surfactant)	400 mg/kg	1	0	Peluso <i>et al.</i> (1998)
Mouse ip exposure (<i>in vivo</i>) alkaline elution of extracted DNA	DNA single-strand breaks	Glyphosate (99.9%)	300 mg/kg	1	0	Bolognesi <i>et al.</i> (1997)
Mouse ip exposure (<i>in vivo</i>) alkaline elution of extracted DNA	DNA single-strand breaks	Roundup (glyphosate 30.4%; 15% surfactant)	270 mg/kg	1	0	Bolognesi <i>et al.</i> (1997)
<i>R. catesbeiana</i> (tadpole)	DNA single-strand breaks; Comet assay	Roundup (glyphosate 30.4%; 15% POEA)	6.75 mg/L	1		Clements <i>et al.</i> (1997)
Mouse ip exposure (<i>in vivo</i>)	8-OHdG	Glyphosate (99.9%)	300 mg/kg	6	0	Bolognesi <i>et al.</i> (1997)

^a Lowest effective dose/highest ineffective dose.

^b 1, positive; 2, negative; 0, not tested.

plate, both with and without metabolic activation (Stegeman and Li, 1990).

Thus, the report of Rank *et al.* (1993) that glyphosate produced an equivocal result for mutagenicity in one bacterial assay is not supported by the other data as shown in Table 2. In the report of Rank *et al.* (1993) the preponderance of the data shows clear evidence of toxicity but no dose response. A single dose exceeded the spontaneous frequency by twofold (without microsomal activation) in TA98. In TA100, a strain that detects base substitution mutations, a single dose also showed a mutational response, but only with S9. Data were pooled from two separate assays, but neither set taken alone satisfied the widely accepted criteria of a positive response (i.e., two consecutive doses to exceed twice the spontaneous frequency). In contrast, the Ames tests completed by Kier *et al.* (1997) at Monsanto using Roundup, Rodeo, and Direct formulations at doses in excess of those reported by Rank *et al.* (1993) were uniformly negative. The studies of Kier *et al.* (1997) were conducted with complete protocols to satisfy international regulatory guidelines for these assays. Accordingly, the findings of Rank *et al.* (1993) must be contrasted with the clear negative responses found by several other investigators. Whether their results were due to the effects of toxicity is uncertain, but the weight of evidence indicates their results represent a false positive result, which is known to occur sporadically

in this and other genotoxicity tests (Brusick *et al.*, 1998).

Other endpoints that detect mutation have been used with Roundup formulations. Differing results were reported for the effect of Roundup in the dominant lethal assay of *Drosophila melanogaster*. One assay carried out using exposure conditions routinely used for this type of study showed no effect of Roundup (Gopalan and Njagi, 1981). A second nonstandard exposure scheme that required chronic exposure (up to 4 days) of larvae until pupation did show a significant elevation of the frequency of sex-linked lethals in spermatocytes (Kale *et al.*, 1995). This was a nonstandard variation of the *Drosophila* sex-linked lethal assay in which every chemical tested was evaluated as positive. Some methodological concerns associated with this report include the authors' lack of experience with the assay, absence of negative controls, and high exposures that included treatment with chemical concentrations that were lethal to half the test population (LC₅₀). No firm conclusions can be made for possible mutagenic effects from Roundup exposure on the basis of these two studies that applied different methodologies.

Chromosomal Aberration Studies

Evaluating the potential for a chemical to cause structural chromosome aberrations provides relevant information for purposes of health risk assessment

since there is a clear association between chromosome rearrangements and cancer (Tucker and Preston, 1996). Virtually all tumors contain structural (and/or numerical) rearrangements (Rabbitts, 1994; Solomon *et al.*, 1991), although these most probably arise late in tumor development. Nevertheless, clear evidence for the production of chromosome abnormalities that are heritable at the cellular level is an important consideration for cancer hazard assessment. As discussed above, results of chronic exposure studies in rats and mice demonstrate that there is no evidence of tumorigenicity for glyphosate, an important fact that should be taken into consideration when evaluating all of chromosomal aberration studies described below.

Glyphosate was negative in an *in vitro* mammalian cytogenetic assay using human lymphocytes with or without microsomal activation at concentrations up to 0.56 mg/mL and at exposures up to 48 h (van de Waart, 1995). These tests were performed according to OECD and EEC guidelines.

Lioi *et al.* (1998a,b), in contrast, have recently reported that glyphosate produced an increased frequency of chromatid breaks as well as other chromosomal aberrations in both cultured human and bovine lymphocytes. There is reason to question these positive results on several grounds. Lioi *et al.* (1998a) reported evidence of chromosomal damage at doses three orders of magnitude lower than the van de Waart (1995) study cited above. Although Lioi *et al.* (1998a) also found that under similar conditions, the fungicide vinclozolin produced similar types and frequencies of chromosomal damage across the same dose range as they reported for glyphosate, vinclozolin is known to produce toxicity by nongenotoxic mechanism(s). In other experiments reported previously by Hrelia *et al.* (1996), the fungicide failed to produce chromosomal aberrations at 70 times the dose applied by Lioi *et al.* (1998a) and failed to show other evidence of direct DNA damage in a number of tests. The treatment protocol of 72 h used by Lioi *et al.* (1998a) was also unusual compared with recognized methodologies. Chemicals that reliably produce chromosomal aberrations in stimulated lymphocytes can do so after a 4-h exposure and often after 20 h of exposure, the usual test intervals. The observation that glyphosate exposures resulted in a reduced growth rate (thus affecting time to first mitosis) is an indication of a toxic effect, and this can have clear implications for the evaluation of any chromosomal aberration data. For an accurate assessment of induced aberration frequency, the cytogenetic evaluations must be conducted in a period of time shortly after exposure (Tucker and Preston, 1996). The results with bovine and human lymphocytes were not consistent. Lioi *et al.* (1998a) found chromosome type breaks in human cells, but few if any with bovine cells (Lioi *et al.*, 1998b), without apparent explanation. Finally, the authors do not explain why under their test conditions

three different chemicals, atrazine, vinclozolin, and glyphosate, produced nearly identical responses over exactly the same dose ranges also in human lymphocytes. This is even more remarkable in view of the findings from other laboratories (Hrelia *et al.*, 1996; van de Waart, 1995) that observed no effects in either glyphosate or vinclozolin at dose levels in excess of 70 times those employed by Lioi *et al.* (1998a).

Glyphosate alone was not active for chromosomal damage (De Marco *et al.*, 1992; Rank *et al.*, 1993). Another study has reported that Roundup can produce chromosomal aberrations in onion root tip cells (Rank *et al.*, 1993). These investigators postulated that the toxic effect of the surfactant in Roundup could be responsible for the effects on the plant cell chromosomes. Goldenboth (1977) found that glyphosate had an effect on water hyacinth root tips and concluded that the dose-dependent effect on the formation of mitotic figures at prolonged exposure times was due to an effect on the spindle apparatus, leading to disorganized chromosomes at anaphase. Given the herbicidal activity of glyphosate, these results are considered secondary to plant toxicity and not relevant to human health.

Of greater relevance than *in vitro* effects is evidence of *in vivo* effects. In this regard, administration of glyphosate to rats did not produce an increase in frequency of chromosomal aberrations (Li and Long, 1988). No effects were observed in rat bone marrow at several time periods posttreatment following intraperitoneal administration of 1.0 g/kg glyphosate.

The *in Vivo* Micronucleus Assay

A number of studies have used the mouse bone marrow micronucleus assay to examine the effects of exposures to glyphosate and Roundup on dividing red blood cells (Table 2). The micronucleus assay targets the most actively dividing cell population of the bone marrow, the polychromatic erythrocytes (PCEs). PCEs represent immature cells in the progression of hematopoiesis to normochromatic erythrocytes (NCEs) found in peripheral blood. The toxic effect of a chemical exposure to bone marrow can be assessed by the ratio of PCE/NCE. Different mechanisms may be involved in the evolution of micronuclei, including chromosome breakage (clastogenesis) or effects on spindle organization (aneuploidogenesis). Almost all the results for either glyphosate or Roundup expressed as micronucleated PCE (MN-PCE) per 1000 PCE fall within the range of control (vehicle) values. The frequency of spontaneously (vehicle) produced micronuclei in newly produced polychromatic erythrocytes was within the historical range for the CD-1 strain of mouse (Salamone and Mavournin, 1994).

All but one of the published or unpublished procedures that have examined the effect of glyphosate or Roundup on the bone marrow have used intraperito-

neal (ip) injection as the route of exposure. While less relevant for purposes of assessing risks for human exposure, ip injection assures high distribution of chemical into the circulatory system of the test species and exposure of target cells in bone marrow with maximum potential for observation of genotoxic events. In the only study done using the more relevant oral route of exposure (NTP, 1992), glyphosate did not produce micronuclei following 13 weeks of dietary administration to B6C3F1 at dosage levels up to 50,000 ppm (11,379 mg/kg body wt/day).

Three studies (Kier *et al.*, 1997) examined the different herbicide formulations containing glyphosate. Rodeo herbicide contains only glyphosate as the IPA salt, while Roundup and Direct are formulations that also contain surfactant systems. These bone marrow micronucleus studies were performed according to accepted EC/OECD guidelines, using ip injection as the route of exposure in CD-1 mice. OECD (1998) guidelines require exposed and control animals (♂/♀ per sex at each dosage and for each time period of exposure) for dosages examined. At least 1000 PCEs per animal were scored for the incidence of MNPCs. In each case, Kier *et al.* (1997) found no evidence of clastogenic effect of the herbicide formulation as measured by an increase in the frequency of PCE-containing micronuclei.

Since Rodeo contains no surfactant, it is therefore less acutely toxic and could be tested at higher dose levels than the other two formulations containing surfactants. The LD₅₀ for ip exposures to Rodeo was calculated to be 4239 mg/kg in CD-1 mice during range-finding experiments. Rodeo exposures for bone marrow micronucleus assays included doses of 3400, 1700, or 850 mg/kg. There was no evidence of micronucleus induction in either males or females at any dose or time point tested, including up to 72 h posttreatment (Kier *et al.*, 1997).

For Roundup, ip exposures in CD-1 mice were up to 86% of the LD₅₀ (643 mg/kg), and bone marrow samples were prepared at 24, 48, and 72 h posttreatment were negative for micronucleus induction (Kier *et al.*, 1997). Roundup exposures at all doses tested up to 555 mg/kg (single dose, ip) failed to produce a significant increased number of MNPCs per 1000 PCE in bone marrow of exposed mice.

A third herbicide formulation using glyphosate and a surfactant was tested in the bone marrow micronucleus assay using CD-1 mice (data not shown in Table 2). The herbicide Direct contains tallow amine surfactant with a longer carbon chain length than POEA, the surfactant used in Roundup. Male and female CD-1 mice were given single ip injections of Direct at three doses; the highest exceeded 80% of the LD₅₀ (436 mg/kg). The doses were 365, 183, and 91 mg/kg of formulation. Bone marrow samples evaluated at 24, 48, and 72 h postexposure were negative for micronucleus induction (Kier *et al.*, 1997). Direct exposures at all doses

tested up to 365 mg/kg (single dose, ip) failed to produce any increase in the number of MNPCs per 1000 PCE in bone marrow of exposed mice when compared to control mice that received saline.

Bolognesi *et al.* (1997) reported that glyphosate and Roundup were weakly positive in the bone marrow micronucleus assay in Swiss/CD-1 mice (Table 2). Roundup (ip) reduced the frequency of PCEs in male mice compared to controls, suggesting some evidence of systemic toxicity. The results of Bolognesi *et al.* (1997) contrast with those of Kier *et al.* (1997) that reported no increased micronucleus formation (even at much higher doses than Bolognesi *et al.* tested). Kier *et al.* (1997) did not see a change in total PCE/NCE ratio among females, but only at the highest dose (3400 mg/kg) when the IPA salt of glyphosate (Rodeo) was used. The protocol used by Bolognesi *et al.* (1997), however, varied from the standard acute bone marrow micronucleus assay and only three or four animals per dose group were used. Two ip injections, each representing half the total dose, were administered 24 h apart. Animals were sacrificed at either 6 or 24 h after the total dose (approximately 48 h after initial exposure). The results reported by Bolognesi *et al.* (1997) are at direct variance with those observed in much larger studies carried out under conditions of accepted GLP. First, they report a significant toxic effect on the bone marrow from exposure to glyphosate compared to controls. The number of PCEs usually decrease with toxicity. The ratio of PCEs to NCEs was 73% in controls, but was reduced to 50% with glyphosate and 30% with Roundup. This frequency of PCE production in control animals is unusual for the Swiss CD-1 mouse (Crebelli *et al.*, 1999) and could be indicative of an elevated level of spontaneous micronucleus production. Kier *et al.* (1997) found that approximate ratios for PCE/NCE were similar for control and treated animals, and this is the general experience for results of a well-conducted test (OECD, 1998). Bolognesi *et al.* (1997) compensated for the use of fewer animals by increasing the total number of cells examined per animal. Thus, Bolognesi *et al.* (1997) relied on counts from 3000 PCEs examined per animal in fewer animals to calculate the frequency of micronuclei per 1000 PCEs in pooled data. This may have skewed results, for example, because one outlier animal would be disproportionately represented. The accepted methodology includes counting PCEs for ♂/♀ animals and requiring increases in at least two. Bolognesi *et al.* (1997) did not provide micronucleus data for individual animals, contrary to customary practice, and presented only summary totals, pooled for all animals.

Rank *et al.* (1993) observed no evidence of significant induction of chromosomal effects in NMRI-Born mice exposed to either glyphosate or Roundup using ip injection. These two materials were administered to male and female mice (♂/♀ per sex at each dose) at dose

levels up to 200 mg/kg body wt. Bone marrow was examined 24 and 48 h after exposure, and cells were scored for NCEs and PCEs as well as for the frequency of MNPCEs. The weighted mean for spontaneous MN/1000 PCE in this strain is 2.06 (range 0.4 to 7.0) (Salamone and Mavourin, 1994). For glyphosate, there was no evidence of increased frequency of micronuclei in the bone marrow and no change in the relative frequency of PCE/NCE. This result is in general agreement with Kier *et al.* (1997).

In summary, there are a large number of *in vivo* bone marrow micronucleus tests that depend on ip exposure to (1) the herbicide Roundup; (2) its active ingredient glyphosate; or (3) the more soluble form of glyphosate as the IPAsalt. These exposures range up to 80% of the LD₅₀ in mice, but have failed to show significant genotoxic effects on replicating bone marrow cells. The bone marrow micronucleus assay is a simple yet reliable method capable of providing evidence for *in vivo* genotoxicity resulting from different mechanisms (Crebelli *et al.*, 1999). The conclusion that must be made from this information is that there are no genotoxic events that occur *in vivo* in the absence of overt bone marrow toxicity. This fact is important in the evaluation of the results of other *in vivo* and *in vitro* results.

In Vitro Sister Chromatid Exchange

Analysis of sister chromatid exchange (SCE) frequency can be an unreliable indicator of genotoxic effect. The frequency of SCE can fluctuate based on osmotic balance. Sodium and potassium chloride concentrations have been implicated in SCE production (Galloway *et al.*, 1987). While somewhat more sensitive than assays of clastogenic activity or chromosomal aberrations, the SCE assay does not indicate a mutagenic effect. Therefore, it is not appropriate to suggest that increases in SCE could be indicative of cancer risk, primarily because of the lack of an associated cellular outcome (Tucker and Preston, 1996). The utility of the *in vitro* SCE assay is questionable, because hazard can be more readily assessed using any number of *in vitro* assays specific for mutation. The SCE assay monitors direct exchange between sister chromatids that suggest recombination. SCE are a cytogenetic manifestation of interchanges between DNA replication products at apparently homologous loci. The exact nature of these exchanges and their relevance to toxic or genetic endpoints are matters of some debate (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). The mechanism of SCE formation has not been established, but it has been suggested that they may involve events closely associated with replication (Tucker and Preston, 1996). Several studies have examined the effects of glyphosate and Roundup on the frequency of SCE in cultured human or animal lymphocytes (Table 2).

Vigfusson and Vyse (1980) were the first to report on

the frequency of SCE in human lymphocyte cultures exposed to Roundup. The authors acknowledged that cytotoxicity was a confounding factor for their results. They observed very minor changes in SCE in lymphocytes from two donors, but only two doses were reported because the highest dose was toxic and no cell growth occurred. Cells from one donor appeared to show a moderate response, but the other did not. Therefore, the results are not internally consistent. Because of this lack of dose response, it is not possible to apply statistical analysis to determine whether or not an observable effect could be described.

Bolognesi *et al.* (1997) reported SCE in cultured human lymphocytes after exposure to glyphosate (1.0 to 6.0 mg/mL) or Roundup (0.1 mg/mL). Glyphosate as the free acid is soluble in this range and has a pH of 2.5. The investigators provided no indication of any precautions taken to ensure against the strong acidity of glyphosate in solution. Glyphosate produced a weak response of about three SCE per cell (estimated from the figure presented) after a 48-h exposure. These results were produced from two donors whose data were pooled (50 metaphases per exposure concentration). Normally, protocols for analysis of cytogenetic data would not permit pooling of data from different individuals or from different experiments. Confidence in results and statistical analysis are only valid when expressed on the basis of the variation of response among the individuals tested. Bolognesi *et al.* (1997) failed to provide the tabulated SCE values for individuals or experiments, so it is quite possible that the variation within the data set explains the apparent increase. According to Bolognesi *et al.* (1997) Roundup was more toxic to lymphocytes, and only doses approximately 10-fold below those tolerated for glyphosate could be tested. Once again, the responses described by these authors are well within the spontaneous SCE frequencies in the human population (see discussion above).

Lioi *et al.* (1998b) reported increases in SCE per cell for bovine lymphocytes exposed to several low doses of glyphosate (up to 29 mg/L). However, changes were not related to exposure over a greater than 10-fold range of dose. Similarly, Lioi *et al.* (1998a) failed to detect a dose response for SCE production in human lymphocytes after exposure to glyphosate. In addition, all of the SCE data reported by Lioi *et al.* (1998a) using either human or bovine lymphocytes were characterized by an extremely low frequency of spontaneous (background) events (e.g., ranging between 1.9 and 2.2 in the human lymphocyte study). More normal values for base SCE frequencies in human lymphocytes range around six per cell. Various values based on data from larger populations have been recorded by Anderson *et al.* (1991) (6.6/cell), Bender *et al.* (1989) (8.0/cell), and the Nordic Study Group (1990) (5±14/cell). This suggests that Lioi *et al.* (1998a,b) could have performed the test

without sufficient scoring experience or that they saw no statistically significant change at any dose.

In Vivo Mutation

In vivo, glyphosate has been shown to be devoid of genotoxic activity in a dominant lethal assay in mice (Wrenn, 1980). This result confirms that there is no reason to suspect that glyphosate could act to effect genetic changes in actively dividing reproductive tissues.

Mutation Studies with AMPA

The available data on AMPA indicate it to be non-genotoxic and nonmutagenic. No mutagenic activity was observed in a *S. typhimurium* mutation test performed on AMPA at concentrations of up to 5000 mg/plate, both with and without an exogenous source of metabolic activation (Shirasu *et al.*, 1980). Similarly, no genotoxic effects were observed in an *in vitro* unscheduled DNA synthesis repair in rat hepatocytes exposed to AMPA at concentrations of up to 5000 mg/mL (Bakke, 1991). *In vivo*, no evidence of micronuclei induction or other chromosomal effects was found in the bone marrow of CD-1 mice treated with AMPA by ip injection at doses of 100 to 1000 mg/kg body wt (Kier and Stegeman, 1993). The results support the weight-of-evidence conclusion that AMPA is nongenotoxic.

DNA-Reactive Species from Glyphosate or Roundup

Glyphosate is not a DNA-reactive chemical. Experiments *in vivo* were carried out in which Swiss CD-1 mice treated by ip administration of glyphosate as the isopropylammonium salt at perilethal doses of 130 and 270 mg/kg (Peluso *et al.*, 1998). Glyphosate administered ip is considerably more toxic than either dermal exposure or by ingestion, and the doses utilized by Peluso *et al.* (1998) should be considered extraordinary. No evidence of DNA adducts was found on examination of kidney and liver from these mice as measured by the ³²P postlabeling procedure. The route of administration should be considered unusual, since ip injection is a route of exposure of little relevance for humans. In mice, the LD₅₀ values are 134 to 545 mg/kg body wt (WHO, 1994a).

When CD-1 mice were exposed ip with a formulation identified as Roundup (600 mg/kg of a 30.4% IPA salt or a dose equivalent to 182 mg/kg body wt) which contained a surfactant, Peluso *et al.* (1998) reported what they described as evidence for DNA adducts by the ³²P postlabeling procedure in tissues isolated after exposure. There are a number of problems with the procedure that led to this conclusion. First, there is no evidence for a dose response over the narrow range of doses examined. Second, the level of adducts reported

is so low that it is well within the range reported for normal endogenous adducts (Gupta and Spencer-Beach, 1996). In addition, it was not determined if the adducts were derived from the formulation ingredients. There is no evidence that direct DNA-reactive intermediates are produced by the surfactants commonly utilized in field formulations of Roundup. The solvent system used to resolve the potential adducts was suitable for the characterization of large, bulky nonpolar polycyclic aromatic hydrocarbon-type nucleotide adducts (Randerath *et al.*, 1984), which are unlike adducts that would be generated from molecules like glyphosate or the surfactant. The poorly resolved adduct spots of the type reported by Peluso *et al.* (1998) are commonly observed in tissues from animals exposed to complex environmental mixtures. In general, exposures to a limited number of chemical components (as might be expected in Roundup) produce well-defined radioactive products on chromatography, unlike the diffuse zones reported. All these considerations suggest that the chromatographic alterations may have been derived from sources other than the formulation ingredients (i.e., naturally occurring molecules or endogenous metabolites). Indeed, Peluso *et al.* (1998) were unable to provide any chemical characterization of the product(s) that they identified as adducts, and it should be concluded that the observations of Peluso *et al.* (1998) are not supportive of a biologically relevant response.

Others have reported that ip injection of Swiss CD-1 mice with glyphosate and Roundup could result in an increased incidence of alkali labile sites in DNA in kidney and liver (Bolognesi *et al.*, 1997). Alkali labile sites are generally produced at basic sites in DNA and may be revealed under conditions that denature DNA secondary structure. The type of assay used by Bolognesi *et al.* (1997) could not differentiate between true abasic sites such as are generated by DNA lyase enzymes, sites produced by excision repair, or natural interruptions in DNA found at points of arrested DNA replication. The effects reported by Bolognesi *et al.* (1997) were observed at 300 mg/kg glyphosate or 900 mg/kg Roundup (this corresponds to 270 mg/kg glyphosate), which are doses close to or in excess of the ip LD₅₀ for mice (WHO, 1994a). DNA breaks could be detected at a brief time after initial exposure, but at 24 h of exposure, there was no evidence of an excess number of alkali labile sites. There are several reasons to question the interpretation of the results from this assay. These include the interpretation of evidence for an increase in single-strand or alkali labile sites. Such breaks might indicate, but could not differentiate between, events due to the increased number of cells arrested in S phase rather than an increase in the number of excision sites. Cytotoxic effects can also be responsible for introduction of single-strand breaks.

Bolognesi *et al.* (1997) reported a dramatic increase

in the number of oxidized guanine, 8-hydroxyguanine (8-OHdG), residues in DNA of liver cells from mice treated with glyphosate, but not Roundup. Opposite results were found for exposures to kidney cells that appeared to accumulate oxidative damage after treatment with Roundup, but not glyphosate. Products of reactive oxygen species, including 8-OHdG, are stable and tend to form adducts with protein and crosslink DNA at lower frequency (Randerath *et al.*, 1997a,b). The findings in the reports of Bolognesi *et al.* (1997) or Peluso *et al.* (1998) are not consistent with a specific mode of action. Increased levels of 8-OHdG residues is not by definition an indicator of chemical-DNA interaction. These products result from secondary effects associated with chemical induction or inhibition of repair of spontaneous lesions due to toxicity. The solvent system utilized by Peluso *et al.* (1998) could not detect oxidation products in DNA (Randerath *et al.*, 1997a). Metabolism studies in rodents have shown that glyphosate is poorly metabolized; therefore, it is unlikely that products of oxidation could be produced directly in the tissues identified as a result of glyphosate exposure as suggested by Bolognesi *et al.* (1997). It could be that toxicity produces reduced repair of spontaneous 8-OHdG that would then lead to an accumulation of oxidation products. Finally, the lack of increased 8-OHdG in the same organs with both glyphosate and Roundup containing the equivalent amount of glyphosate suggests that glyphosate is not causing the change observed.

Other assays have been used to indirectly demonstrate the possibility of formation of DNA-reactive species from exposure to Roundup. Direct reaction with purine or pyrimidine nucleotides could lead to elimination of an altered base on exposure to alkali. Alkali-sensitive sites resulting from depurination or depyrimidation events can be detected in the Comet assay, a methodology to demonstrate DNA strand breaks. Clements *et al.* (1997) used the Comet assay to examine DNA in erythrocytes from tadpoles exposed to various herbicides including Roundup. Clements *et al.* (1997) reported evidence of a treatment-related increase in DNA breaks as measured by migration of DNA from the bulk of nuclear material in an electrophoretic field. Tadpole erythrocytes were unaffected at the lowest concentration of Roundup diluted in water (1.7 mg/mL), but at greater concentrations (6.75 or 27 mg/mL) did produce evidence of single-strand breaks (SSB) in alkaline Comet assays. The dose of Roundup formulation used in these assays was considerably greater than would be expected at environmental concentrations. Tadpoles were bathed in the exposure concentrations for a period of 24 h prior to testing. Other tests have clearly shown that glyphosate does not interact with DNA directly, so the effects observed may be from secondary effects of cytotoxicity. Although efforts were taken (trypan blue exclusion) to select cells

not undergoing necrosis or autodigestion of DNA, cytotoxicity may have been unavoidable at the doses utilized in the assay.

Rat primary hepatocyte cultures showed no evidence of an increase in unscheduled DNA synthesis (UDS) after a wide range of exposures to glyphosate *in vitro*. Doses examined ranged over 3 orders of magnitude but failed to produce evidence of DNA repair (Li and Long, 1988). These observations in a well-characterized and sensitive system indicate an absence of DNA reactivity, either direct or following hepatocellular biotransformation (Williams *et al.*, 1989).

Evaluating Genotoxicity Data: Weight-of-Evidence Approach

When evaluating data for genotoxicity, a primary goal is to determine (a) the likelihood of occurrence of a key event; and (b) whether that event might lead to heritable changes associated any adverse effect *in vivo*, including cancer. The basis upon which a weight-of-evidence evaluation can be constructed include the following:

- Any statistically significant observations should be reproducible and biologically significant.
- A dose-response relationship should exist for effects.
- The effects should be permanent and progressive, as opposed to reversing upon cessation of chemical dosing.
- The nature of DNA effects should be characterized.
- The database should be consistent or inconsistencies adequately explained.
- The effects produced in the assay should be relevant to humans.

A central objective of the weight-of-evidence is to avoid a situation that could permit one experimental test result to be accorded greater weight over others. A conceptual approach to the relative weighting of genotoxicity testing data in the final assessment of mutagenic or carcinogenic potential is shown in Fig. 3. This model is based on the National Research Council guidance to evaluating sources of data for risk evaluation (NRC, 1983) and is similar to procedures recommended by several regulatory agencies (e.g., U.S. EPA, 1996b, ^a Proposed Guidelines for Carcinogen Risk Assessment^o) for mutagenicity risk assessment.

The key features of the weight-of-evidence scheme described in Fig. 3 are its ability to accommodate results from multiple testing protocols and its requirement to place a premium on consistency and coherence of results. Greater weight is given to results from laboratories using accepted, well-validated protocols employing GLP procedures. The scheme can also function as a tool for analysis of a specific protocol, evaluating internal consistency of results from testing for similar

Guidance for preparing a Weight-of-Evidence analysis for mutagenicity data for a chemical.

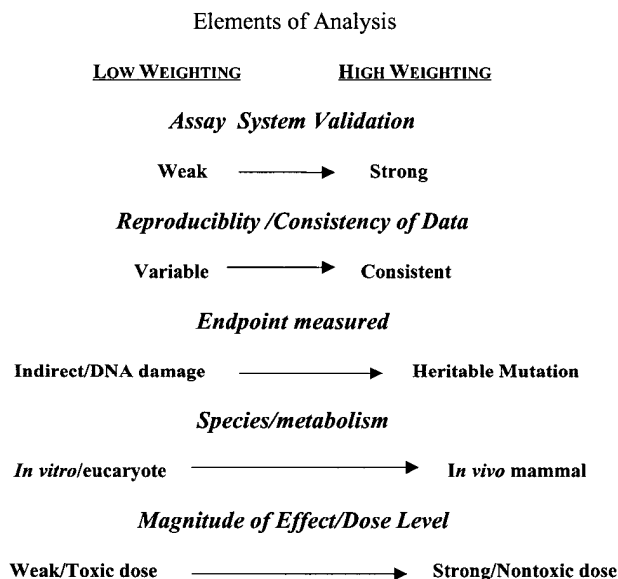


FIG. 3. Weight-of-evidence data hierarchy organization for evaluation and preparation of a statement of the potential for mutagenic activity of a compound.

endpoints. On the other hand, a result from a novel procedure might be acceptable because it is deemed to provide important evidence of a chemical mode of action.

The weight-of-evidence analysis is also significantly affected by the relevance of the data available. Short-term assays disclose evidence of genotoxic events *in vitro* or *in vivo* that can be compared to more comprehensive examinations of animals such as by the 2-year rodent cancer bioassay. For purposes of human hazard assessment, greater confidence should be placed in those test systems that examine possible genetic effects from chemical exposure of animals than in tests that rely on selected homogeneous cell populations raised and tested *in vitro*. Chemical exposures of biological systems carried out *in vitro* are much less realistic, and results of such tests can be determined by the effects of toxicity. Such toxicity can occur at unusually high exposure concentrations and/or be dependent on metabolic and detoxication capabilities. Finally, a weight-of-evidence evaluation seeks to establish a dose-response relationship. Greater attention should be given wherever there is a clear association between increased exposure and a genetic effect.

Weight-of-Evidence Narrative

The database for genetic effects of glyphosate and Roundup is both large and heterogeneous. Such extensive data sets are sometimes problematic to interpret,

but this is not the case for glyphosate. Sporadic positive responses (i.e., nonreproducing) are inherent within assays used to detect mutagenicity or genetic alterations, particularly *in vitro* tests (Brusick *et al.*, 1998; Kirkland and Dean, 1994). Scientific objectivity precludes emphasis on a few of positive responses rather than the overall response pattern and trend of the results.

Many testing schemes for mutagenicity and other short-term assays are conducted using acute exposure protocols designed for purposes of cancer hazard identification. In the case of glyphosate, there are no tumorigenic endpoints in rodents, or other animals that have been tested, and hence there is no cancer hazard to attribute to any genotoxicity ending.

The information in Table 2 clearly shows that in diverse test systems, glyphosate alone, or as a formulation in Roundup fails to produce any evidence for mutation induction. Effects of glyphosate on chromosomal organization *in vivo* have been almost wholly negative. The micronucleus data (Table 2) and those for chromosomal effects in bone marrow (Li and Long, 1988) are consistently negative except for the micronucleus data from Bolognesi *et al.* (1997), which must be viewed with reservation until a more complete description of the data is available. The remainder of animal studies carried out *in vivo* show no effect of either glyphosate or Roundup. On the other hand, the results of *in vitro* chromosomal aberration tests are more mixed. For reasons described above, it is difficult to give equal weight to the studies based on the quality of the study data presented. In particular, the two studies on bovine and human lymphocytes presented by Lioi *et al.* (1998a,b) are inadequate and, as described, have many problems relating to the internal consistency of the data for other pesticides tested. Accordingly, these studies are not weighted equally with the assay carried out under GLP conditions (van de Waart, 1995).

There is evidence for the production of effects such as single-strand breaks in DNA, but none of these have been linked to the presence of identifiable adducts and are therefore most likely due to secondary effects of toxicity. Metabolic studies in rodents plainly show that greater than 99% of glyphosate is rapidly excreted unchanged, and there is very little evidence that chemical residues are associated with any tissue. Bolognesi *et al.* (1997) have reported evidence of accumulation of 8-OHdG adducts in livers of mice treated with glyphosate ip, but this cannot be reconciled with the fact that glyphosate is not metabolized. There has been absolutely no evidence produced to date that shows glyphosate or Roundup is directly responsible for these events. It may be that the injection of such a large quantity of glyphosate (2–3–150 mg) creates stress-related events that lead to accumulation of these oxidative adducts, which do occur spontaneously. Similarly, the apparent production of single-strand breaks

in liver or renal tissue DNA (Bolognesi *et al.*, 1997; Peluso *et al.*, 1998) after alkaline elution experiments could also be indicative of events of cytotoxicity that reduces or retards rates of DNA replication, giving the appearance of breakage events. The fact that these events were transitory, being no longer evident 24 h after exposure also suggests an indirect effect of exposure. Also, the negative UDS assay in hepatocytes (Li and Long, 1988) would tend to confirm that the SSB of Bolognesi *et al.* (1997) likely occur in S phase. Finally, Clements *et al.* (1997) also appear to have found a weak effect of Roundup on integrity of tadpole erythrocyte DNA in the Comet assay. Once again, the nature of the exposure conditions and the concentrations used were considerably greater than might be expected from environmental exposures. Peluso *et al.* (1998) could detect no evidence of DNA adducts or covalently bound residues in DNA from tissues of mice exposed to glyphosate alone. The weak production of SSB shown by alkaline elution and by the alkaline Comet assay (Clements *et al.*, 1997; Bolognesi *et al.*, 1997; Peluso *et al.*, 1998) are all suggestive of secondary effects of glyphosate exposure and probably arise from cytotoxicity rather than any direct effect of exposure.

The data relating to SCE production presented by Lioi *et al.* (1998a,b) and Bolognesi *et al.* (1997) are questionable on both methodological and scientific grounds. The spontaneous frequency of SCE in untreated cells was extremely low compared with the norm for human lymphocytes, the number of individuals whose lymphocytes were examined does not meet any standard for determining statistical significance, and the size of the increases observed was variable and not always dose related. Finally, the levels observed were well within the accepted variation for the incidence of SCE in the human population.

It is concluded that on a weight-of-evidence analysis of the data for glyphosate and for Roundup that they are neither mutagenic nor genotoxic as a consequence of a direct chemical reaction with DNA. The assay systems used in short-term genotoxicity tests are extremely sensitive, but no single test is sufficient to form the basis for conclusive proof of evidence of a genotoxic effect. In the case of these compounds, there is evidence that in circumstances that lead to cytotoxicity (i.e., high-dose experimental conditions), as would be predicted for any chemical that undergoes such testing, some effect may be observed such as the production of single-strand breaks. The balance of the credible data from *in vitro* and *in vivo* test results confirm the safety of glyphosate and Roundup as nongenotoxic and conforms to the fact that glyphosate is noncarcinogenic.

Summary

The potential genotoxicity of glyphosate has been tested in a wide variety of *in vitro* and *in vivo*

assays. No genotoxic activity was observed in standard assays conducted according to international guidelines. These assays include the *S. typhimurium* (Ames assay) and *E. coli* WP-2 reversion assays, recombination (rec-assay) with *Bacillus subtilis*, Chinese hamster ovary cell gene mutation assay, hepatocyte primary culture/DNA repair assay, and *in vivo* mouse bone marrow micronucleus and rat bone marrow cytogenetics assays. Recently, investigators have reported evidence of genotoxic effects in a limited number of studies. However, these assays used toxic dose levels, irrelevant endpoints/test systems, and/or deficient testing methodology. In view of the clear negative responses in relevant, well-validated assays conducted under accepted conditions, it is concluded that glyphosate is neither mutagenic nor clastogenic. On the basis of this evaluation, glyphosate does not pose a risk for production of heritable or somatic mutations in humans.

The mutagenic potential of Roundup herbicide and the POEA surfactant has been evaluated in several bacterial mutagenicity assays. While a marginal response was reported in one limited investigation, results from other complete, replicated studies conducted according to international guidelines and Good Laboratory Practices show that these materials are not mutagenic. Glyphosate herbicide formulations and the POEA surfactant have been evaluated for the ability to produce chromosomal aberrations in several mouse micronucleus assays as well as investigations with onion root tip cells and *Drosophila*. It is concluded that these materials were not mutagenic in mice. Results from the nonmammalian assays were confounded by various factors and provided no biologically relevant evidence of genotoxicity. DNA interaction studies with Roundup herbicide have been reported in the literature. While some of these studies reported positive effects, methodological limitations render the data scientifically uninterpretable and unacceptable for safety assessment. For example, the positive effects were observed only at cytotoxic concentrations *in vitro* and at perilethal doses *in vivo* administered by an irrelevant route of exposure (i.e., ip injections). Thus, the changes occurred only under extreme conditions of exposure in assays that do not directly assess mutagenicity and are known to produce effects that are secondary to toxicity. It is believed that the high, unrealistic dose levels used in these studies were sufficiently toxic to produce secondary effects rather than direct genotoxicity. In view of all this information, Roundup is not considered to be mutagenic under conditions that are relevant to animals or humans.

EVALUATION OF POTENTIAL SPECIFIC ORGAN/SYSTEM EFFECTS

Salivary Gland Changes

When salivary gland alterations were observed in rats and mice following subchronic glyphosate admin-

istration, additional research was undertaken to investigate the mechanism by which this change occurred (NTP, 1992). It was hypothesized that glyphosate produced the alterations via weak β -adrenergic activity. However, careful examination of the data and consideration of other factors do not support this hypothesis.

In a follow-up study conducted by NTP (1992), male rats were fed glyphosate for 14 days at a dietary level of 50,000 ppm, which was the high-dose level from the subchronic study, while other rats were given isoproterenol (a β -adrenergic agonist). Both compounds produced increased salivary gland weights. When isoproterenol was given with propranolol, a β -blocker, there was no increase in salivary gland weight. In contrast, salivary gland weights remained elevated when propranolol was administered along with glyphosate, although the elevation was not as high as that seen when glyphosate was administered alone. The inability of a β -blocker to significantly inhibit the effects of glyphosate indicates that it does not act as a β -agonist.

Other factors were considered to help resolve questions of salivary gland effects and causality. First, if glyphosate was a β -agonist material, its effect would be to stimulate β -receptors in other effector organs and produce a characteristic set of cardiocirculatory effects such as increased heart rate and cardiac output as well as decreased blood pressure and peripheral resistance. None of these effects were noted in two pharmacology studies in which glyphosate was administered intravenously to dogs and rabbits (Tai *et al.*, 1990; Takahashi, 1992). Similarly, it is known that isoproterenol and other β -agonists cause myocardial necrosis (Lockett, 1965) and enlargement of heart ventricles (Schneyer, 1962) following prolonged treatment. Glyphosate did not produce any effects in heart tissue, even after chronic exposure at very high doses, providing additional support to the argument that glyphosate does not act as a β -agonist. Furthermore, glyphosate is not structurally related to known β -agonists. It is concluded that glyphosate has no significant β -adrenergic activity and therefore could not produce salivary gland changes via β -agonist activity.

Indeed, there are a number of other potential mechanisms of salivary gland alteration, including nonchemical modes of action. For example, salivary gland secretion has been shown to be affected by the texture and moistness of feed (Jackson and Blackwell, 1988), and salivary gland enlargement has been caused by malnutrition. Glyphosate could be acting by such a nonchemical mechanism. Because glyphosate is a strong organic acid, dietary administration at relatively high levels may cause mild oral irritation leading to increased salivary gland size and flow. In the chronic exposure studies of glyphosate there were several salivary gland changes. These changes were: (1) most pronounced in the parotid gland, responsible for secretion of serous fluid in response to such stimuli as acidic

materials; (2) absent in the sublingual gland that releases mucous fluid in response to other stimuli; and (3) observed to an intermediate degree in the submandibular gland that contains a mixture of mucous and serous secreting cells. This pattern of observations is consistent with the hypothesis that the salivary gland change observed are a biological response to the acidic nature of glyphosate.

Regardless of the mechanism involved, there are several reasons to conclude that the salivary gland change observed is of doubtful toxicological significance. The change occurred in the absence of other significant adverse effects, indicating that the health of the animals was not adversely impacted. Furthermore, the salivary gland alteration was not associated with any adverse clinical or pathological effect even in chronic studies. Such alteration cannot be considered preneoplastic because the tumor rate was not increased in chronic bioassays. These salivary gland changes are not known to represent any pathologic condition and have no relevance to humans. Therefore, the finding is not considered to be either toxicologically significant or adverse.

Potential for Endocrine Modulation

The U.S. Environmental Protection Agency has developed a two-tiered screening and testing strategy for evaluating the endocrine modulating potential of environmental substances. Tier I screening assays include both *in vitro* and short-term *in vivo* assays designed to detect substances with the ability to interact with the endocrine system. Tier II tests include long-term *in vivo* multigeneration reproductive toxicity tests that more definitively determine and characterize any endocrine modulating effects for subsequent risk assessment. In addition to efforts within the United States, other countries, led primarily by Japan and the OECD (Office of Economic and Development) member countries, are developing similar *in vitro* and *in vivo* approaches to assess chemicals for endocrine activity.

In Vitro Assays

A number of *in vitro* assays have been developed to assess potential endocrine modulating effects of a chemical. The primary use of these *in vitro* assays in hazard identification is to screen large numbers of chemicals and to determine which ones should be further studied in more definitive *in vivo* testing. As with any screening strategy, these assays are generally designed such that any errors are likely to be false positives rather than false negatives. When a positive result is reported in these assays, *in vivo* work is indicated to confirm, characterize, and quantify the true nature of the endocrine-modulating properties of the chemical. The recent concern over endocrine modulation and the availability of inexpensive screens is

leading to the testing of chemicals in these *in vitro* assays regardless of the size and reliability of the more definitive *in vivo* database.

Petit *et al.* (1997) tested glyphosate and 48 other chemicals in two complementary assays: one measuring activation of the estrogen receptor from rainbow trout in a yeast system and the other evaluating vitellogenin production in a trout liver cell culture system. Glyphosate had no estrogenic activity in either assay.

In Vivo Studies

The repeat dose *in vivo* toxicology studies required by the U.S. EPA and other worldwide regulatory agencies detect modulation of endocrine system activity (Carney *et al.*, 1997; Stevens *et al.*, 1997, 1998). These studies are more predictive than *in vitro* screening assays as they assess a variety of endocrine-sensitive endpoints in animals that are capable of metabolic activation and/or detoxication. These studies also use extended exposure periods encompassing various stages of endocrine development. Endocrine-active substances affecting a single or multiple endocrine target sites invariably initiate direct or compensatory biochemical, cellular, and/or histopathological processes which will be detected in standard toxicology studies required for pesticide registration in Canada, Europe, Japan, and the United States. A comprehensive histopathological assessment of endocrine tissues combined with gross organ pathology and organ weight data allows detection of all adverse endocrinopathies.

The standard toxicology studies that provide valuable information on potential endocrine-modulating effects include subchronic, chronic, developmental, and reproduction studies. The multigeneration rat reproduction study is the most definitive study for evaluating the potential of substances to produce endocrine-modulating effects in humans and other mammals (U.S. EPA, 1998b). This study evaluates effects on gonadal development/function, estrous cycles, mating behavior, fertilization, implantation, *in utero* development, parturition, lactation, and the offsprings' ability to survive, develop, and successfully reproduce. A comprehensive histopathological assessment of all major organ systems also is a prominent feature of these studies. Developmental toxicity studies evaluate effects on many of these same processes, while subchronic and chronic studies incorporate numerous direct and indirect evaluations of endocrine and reproductive tissues such as target organ weights and a comprehensive assessment of endocrine organ pathology.

There were no definitive findings in the subchronic, chronic, developmental, or reproductive toxicity studies indicating that glyphosate or AMPA produced any endocrine-modulating effects (see Tables 3 and 4). Histopathological observations of endocrine and reproduc-

tive tissues from animals in a chronic and a two-generation toxicity study are presented in Tables 3 and 4 to illustrate the magnitude and comprehensive nature of these assessments. The data clearly indicate that glyphosate exposure had no adverse histological consequence on any reproductive or endocrine tissue from either male or female rats even at exaggerated dosage levels. Negative results also were obtained in a dominant lethal study conducted at very high doses. While this latter test is typically used to assess genetic toxicity, substances that affect male reproductive function through endocrine modulating mechanisms can also produce effects in this type of study. To summarize, no effects were observed in two independent, multigeneration reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and AMPA are not reproductive toxicants and do not perturb the endocrine system. The U.S. EPA (1998a) reviewed these studies and also concluded that there was no evidence to suggest that glyphosate produces endocrine-modulating effects.

The results of subchronic and developmental toxicity tests on POEA also showed no evidence of endocrine modulation. In addition, the metabolism of POEA would be expected to produce short-chain carboxylic acids and similar derivatives, which are not considered to be endocrine modulators. The lack of any indications of hormonal activity in subchronic toxicity studies with Roundup herbicide supports the conclusion that POEA does not possess endocrine modulating activity.

Summary

The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies. The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies. Results from standard studies with AMPA, Roundup herbicide, and the POEA surfactant also failed to show any effects indicative of endocrine modulation. Therefore, it is concluded that the use of Roundup herbicide has no potential to produce adverse effects on endocrine systems in humans nor in other mammals.

Potential for Neurotoxicity

As discussed above, glyphosate, AMPA, POEA, and Roundup herbicide have been tested in numerous subchronic, chronic, and reproductive toxicity studies. In

TABLE 3
Summary Incidence of Microscopic Findings in Reproductive and Endocrine Organs
in a 2-Year Rat Study with Glyphosate^a

Dose levels (ppm)	0	2000	8000	20,000
Epididymis(-ides)				
Decrease/absence of sperm	12 (60) ^b	14 (60)	17 (60)	19 (60)
Granuloma, sperm	1 (60)	0 (60)	1 (60)	0 (60)
Atrophy	1 (60)	0 (60)	0 (60)	0 (60)
Hyperplasia, ductal epithelium	0 (60)	0 (60)	1 (60)	1 (60)
Testis(-es)				
Degeneration/atrophy, seminiferous tubules, bilateral	14 (60)	16 (60)	14 (60)	22 (60)
Arteritis/periarteritis	17 (60)	12 (60)	18 (60)	21 (60)
Hyperplasia, interstitial cells	1 (60)	1 (60)	0 (60)	1 (60)
Spermatocoele	1 (60)	0 (60)	0 (60)	0 (60)
Interstitial cell tumor	2 (60)	0 (60)	3 (60)	2 (60)
Granuloma, spermatoc	0 (60)	1 (60)	0 (60)	1 (60)
Degeneration/atrophy, seminiferous tubules	6 (60)	8 (60)	8 (60)	8 (60)
Ovaries				
Cyst(s), follicular	13 (60)	7 (60)	8 (60)	9 (59)
Cyst(s), paraovarian bursa	0 (60)	1 (60)	1 (60)	1 (59)
Granulosa cell tumor	0 (60)	2 (60)	1 (60)	0 (59)
Lymphoma in@trate	0 (60)	0 (60)	0 (60)	1 (59)
Theca cell tumor	1 (60)	0 (60)	0 (60)	0 (59)
Arteritis/periarteritis	0 (60)	0 (60)	1 (60)	0 (59)
Metastatic cortical carcinoma, adrenal	0 (60)	0 (60)	0 (60)	1 (59)
Uterus				
Dilatation, endometrial glands	7 (60)	6 (60)	5 (60)	3 (59)
Squamous metaplasia, endometrial glands	6 (60)	2 (60)	1 (60)	2 (59)
Inflammation, endometrium	0 (60)	1 (60)	2 (60)	2 (59)
Dilation of uterine lumen (hydrometra)	7 (60)	9 (60)	16 (60)	8 (59)
Hyperplasia, endometrial glands	0 (60)	0 (60)	2 (60)	3 (59)
Hypertrophy/hyperplasia, endometrial stroma	1 (60)	0 (60)	0 (60)	1 (59)
Prostate				
In@trate, mononuclear/lymphocytic, interstitial	3 (60)	0 (60)	1 (60)	1 (60)
Inflammation	11 (60)	14 (60)	16 (60)	16 (60)
Hyperplasia, acinar epithelium	2 (60)	4 (60)	1 (60)	4 (60)
Adenocarcinoma	1 (60)	0 (60)	0 (60)	0 (60)
Atrophy	1 (60)	2 (60)	0 (60)	2 (60)
Mucoid epithelial metaplasia	0 (60)	1 (60)	1 (60)	1 (60)
Cyst	0 (60)	0 (60)	1 (60)	0 (60)
Seminal vesicle(s)				
Inflammation	2 (60)	3 (60)	3 (60)	3 (60)
Atrophy	11 (60)	5 (60)	12 (60)	13 (60)
Distended with secretion	2 (60)	0 (60)	0 (60)	0 (60)
Inflammation, coagulation gland	1 (60)	5 (60)	1 (60)	2 (60)
Secretion decreased	0 (60)	2 (60)	0 (60)	1 (60)
Hyperplasia, epithelium	0 (60)	1 (60)	1 (60)	0 (60)
Pituitary				
Adenoma, pars distalis	34 m (60)	32 m (58)	34 m (58)	31 m (59)
	45 f (60)	48 f (60)	46 f (60)	34 f (59)
Hyperplasia, pars distalis	10 m (60)	10 m (58)	9 m (58)	10 m (59)
	6 f (60)	7 f (60)	7 f (60)	8 f (59)
Vacuolation, pituicytes	0 m (60)	0 m (58)	0 m (58)	1 m (59)
	0 f (60)	0 f (60)	2 f (60)	1 f (59)
Mammary gland				
Adenoma/adenoma/broma/broma	0 m (43)	1 m (31)	1 m (41)	1 m (37)
	25 f (58)	24 f (54)	27 f (59)	28 f (57)
Galactocoele(s)	3 m (43)	3 m (31)	2 m (41)	2 m (37)
	8 f (58)	14 f (54)	4 f (59)	9 f (57)
Prominent secretory activity	6 m (43)	8 m (31)	11 m (41)	5 m (37)
	29 f (58)	26 f (54)	28 f (59)	28 f (57)
Hyperplasia	0 m (43)	2 m (31)	2 m (41)	0 m (37)
	16 f (58)	19 f (54)	13 f (59)	22 f (57)
Carcinoma/adenocarcinoma	1 m (43)	0 m (31)	0 m (41)	0 m (37)
	13 f (58)	10 f (54)	14 f (59)	9 f (57)

TABLE 3D Continued

Dose levels (ppm)	0	2000	8000	20,000
Adenocarcinoma	0 m (43)	0 m (31)	0 m (41)	1 m (37)
Inflammation, granulomatous	0 f (58)	1 f (54)	0 f (59)	1 f (57)
Inflammation, chronic	1 m (43)	0 m (31)	0 m (41)	0 m (37)
	0 f (58)	1 f (54)	0 f (59)	0 f (57)
Fibrosis	0 f (58)	1 f (54)	0 f (59)	0 f (57)
Carcinoma	1 f (58)	0 f (54)	0 f (59)	1 f (57)
Thyroid				
Hyperplasia/cystic hyperplasia, follicular epithelium	4 m (60)	2 m (58)	1 m (58)	2 m (60)
	1 f (60)	1 f (60)	0 f (60)	3 f (60)
C cell adenoma	2 m (60)	4 m (58)	8 m (58)	7 m (60)
	2 f (60)	2 f (60)	6 f (60)	6 f (60)
C cell hyperplasia	5 m (60)	1 m (58)	6 m (58)	5 m (60)
	10 f (60)	5 f (60)	9 f (60)	5 f (60)
Follicular cyst(s)	2 m (60)	1 m (58)	3 m (58)	3 m (60)
	2 f (60)	1 f (60)	0 f (60)	1 f (60)
C cell carcinoma	0 m (60)	2 m (58)	0 m (58)	1 m (60)
	0 f (60)	0 f (60)	1 f (60)	0 f (60)

Note. m, males; f, females.

^a Data from Stout and Ruecker (1990).

^b All deaths reported. Incidence (total number of animals examined).

another study, the IPA salt of glyphosate was administered to dogs for 6 months (Reyna and Thake, 1983). The design of all these studies included a number of parameters that evaluate the potential of these materials to produce neurotoxicity. Histopathologic examinations were routinely conducted on brain, spinal cord, and peripheral nervous tissues such as the sciatic nerve. In addition, the animals in these studies were regularly observed for unusual clinical signs of toxicity that would indicate any functional effect on the nervous system. The developmental toxicity studies conducted with glyphosate, AMPA, and POEA included examinations to determine if there were adverse effects in the developing nervous system. There was no evidence of neurotoxicity in any of these studies.

Roundup was administered to beagle dogs as a single oral dose at levels of 59 and 366 mg/kg (Naylor, 1988). Animals were continuously observed for 2 to 3 h after dosing for clinical signs of toxicity. A detailed neurological examination consisting of 12 different measurements of spinal, postural, supporting, and consensual reflexes was performed before treatment, during the postadministration observation period, and again on the following day. Reflexes appeared normal, and there were no clinical signs indicative of neuromuscular abnormalities.

It is concluded that there was no evidence of neurotoxicity in any of the toxicology studies even at very high doses. The U.S. EPA has evaluated all the data with glyphosate and also reached this conclusion (U.S. EPA, 1998a). It was also noted by the Agency that no neuropathy or alterations were seen in the fetal nervous system in the developmental and reproductive toxicology studies.

The Potential for Synergistic Interactions

Herbicides are often applied in combination with other active ingredients and/or surfactants. This has raised the question of possible synergistic interactions (i.e., more than additive response) between these materials. It is noteworthy that studies published in the scientific literature, including a comprehensive study of more than 400 combinations of pesticides, have shown that synergism is rare (Carpenter *et al.*, 1961; Keplinger and Deichmann, 1967; Federation of German Research Societies, 1975; Groten *et al.*, 1997). The toxicity of glyphosate has been evaluated in combination with several surfactants and/or other herbicides in acute studies with rats and aquatic species. Based on the results of these studies, it is concluded that the simultaneous exposure of glyphosate and other materials does not produce a synergistic response.

Data that fail to demonstrate evidence for synergism between weakly estrogenic chemicals by the absence of the production of greater response to mixtures have been presented by various investigators. In a study conducted by Baba *et al.* (1989), oral LD₅₀s were determined in rats for each component of Roundup herbicide. The interactions were evaluated by the graphic method of Shirasu *et al.* (1978), and ratios were calculated using Finney's equation. It was concluded that the interaction between glyphosate and the POEA surfactant was antagonistic rather than synergistic. Heydens and Farmer (1997) used the harmonic mean formula of Finney to compare the ^a expected^o and ^a observed LD₅₀ and LC₅₀ values for rats and aquatic species exposed to several combinations of glyphosate with other herbicides and/or surfactants. None of the

TABLE 4
Summary of Reproductive and Microscopic Findings in a Two-Generation Rat
Reproduction Study with Glyphosate^a

Dose levels (ppm):	0			30,000		
	FO	F1A	F1A-remate	FO	F1A	F1A-remate
Total paired females	30	30	30	30	30	30
Females with confirmed copulation/total paired	96.7%	100.0%	83.3%	100.0%	96.7%	86.7%
Pregnant/total paired	80.0%	93.3%	53.3%	93.3%	86.7%	83.3%
Pregnant/confirmed copulation	82.8%	93.3%	64.0%	93.3%	89.7%	96.2%
Males with confirmed copulation/total paired	86.7%	93.3%	70.0%	90.0%	83.3%	80.0%
Males impregnating females/total paired	70%	90.0%	46.7%	83.3%	80.0%	76.7%
Males impregnating females/confirmed copulation	80.8%	96.4%	66.7%	92.6%	96.0%	95.8%
Precoital length for pregnant animals (days)	3.6	2.8	3.7	3.7	3.2	2.5
Gestational length (days)	22.3	22.4	22.4	22.3	22.6	22.5
Litter size						
Female	6.7	6.6	6.0	5.7	5.5	5.6
Male	6.6	5.4	5.9	5.8	5.3	5.2
Combined	13.3	12.0	11.9	11.5	10.8	10.7
Terminal body weight (g)						
Males	549.6	625.0		503.5*	543.4*	
Females	296.3	316.2		265.9*	284.8*	
Organ weights (g)						
Ovary(-ies)	0.1343	0.1579		0.1269	0.1587	
testis(-es)	5.9959	6.6090		5.7905	6.3857	
Histopathology of tissue/organs						
Epididymis(-ides)						
Vacuolation, duct epithelium	1 (30) ^b					
Inflammation, mononuclear, interstitial		1 (30)		5 (30)		
Chronic inflammation, fibrosis					1 (29)	
Periepididymal adipose tissue, inflammation, granulomatous					1 (29)	
Hypospermia, unilateral					1 (29)	
Testis						
Hypoplasia/atrophy seminiferous tubule, bilateral	2 (30)	1 (30)		1 (30)		
Degeneration seminiferous tubules, unilateral		1 (30)			1 (29)	
Hemorrhage		1 (30)				
Granuloma, spermatic					1 (29)	
Ovary(-ies)						
Cyst(s)		3 (30)		1 (30)	3 (30)	
Inactive		1 (30)				
Uterus						
Remnant, implantation site	10 (29)	11 (29)		7 (29)	13 (29)	
Mesometrium, calcified						
implantation remnant	1 (29)					
Dilation of uterine lumen (hydrometra)	5 (29)	5 (29)		9 (29)	7 (29)	
Pigment deposition		3 (29)			7 (29)	
Mononuclear infiltrate endometrium		1 (29)			1 (29)	
Vascular necrosis mesometrium		1 (29)				
Vagina						
Mononuclear cell infiltrate					1 (29)	
Prostate						
Chronic inflammation	14 (30)	4 (29)		12 (30)		
Mononuclear cell infiltrate		1 (29)			1 (29)	
Edema		2 (29)				
Seminal vesicle						
Mononuclear cell infiltrate		1 (29)			1 (29)	

TABLE 4D Continued

Dose levels (ppm):	0			30,000		
	FO	F1A	F1A-remate	FO	F1A	F1A-remate
Pituitary Cyst(s)		2 m (30) 2 f (30)			2 m (28) 3 f (23)	
Adenoma, pars distalis		1 f (30)				
Mammary gland Galactocoele		1 f (28)				
Mononuclear cell, in@trate		1 m (25)			1 f (30)	

Note. Significantly different from control, **P* # 0.01. m, males; f, females.

^a Data from Reyna (1990).

^b Incidence (total number of animals examined).

combinations showed any evidence of synergism. Martinez and Brown (1991) studied the interaction between glyphosate and POEA administered intratracheally to rats at very high dose levels. Based on the resulting pulmonary damage and mortality data, the authors concluded that a synergistic response occurred. However, no supporting mathematical analysis or other basis for the conclusion was presented. In a similar study, Adam *et al.* (1997) investigated the oral and intratracheal toxicity of POEA, glyphosate, and Roundup herbicide. In contrast to the conclusions of Martinez and Brown, these authors concluded that there appeared to be no synergism with glyphosate and POEA. In conclusion, there is no reliable evidence indicating synergistic interactions between glyphosate and other materials.

HUMAN EXPERIENCE

Irritation Studies

Dermal irritation studies with Roundup herbicide in human volunteers have shown, at most, only mild effects. In two separate studies, exposure to Roundup at a normal spray dilution (; 0.9% glyphosate as the IPA salt, IPAG) or at a higher concentration (; 4.1% IPAG) produced no skin irritation or sensitization when applied for 24 h (Shelanski, 1973). Maibach (1986) evaluated Roundup and commonly used household products (Johnson & Johnson baby shampoo, Ivory dishwashing detergent, and Pinesol liquid cleaner) for acute irritation, cumulative irritation, and photoirritation, as well as allergic and photoallergic activity. Mild irritation was observed in a few individuals as a result of application of concentrated product directly to skin for 24 h; however, no dermal sensitization, photoirritation, or photosensitization was observed. The authors concluded that Roundup herbicide and the baby shampoo had less irritant potential than either the cleaner or dishwashing detergent. There was no difference between Roundup and the baby shampoo in terms of irritation potential.

Occupational Exposure

One controlled study that investigated the potential effects of Roundup exposure in applicators has been reported in the scientific literature. The remaining information involves reports of effects from individuals following use of the product. These include data gathered by the State of California and three published studies.

Jauhiainen *et al.* (1991) evaluated the short-term effects of glyphosate exposure in agricultural herbicide applicators. Data from applicators who sprayed Roundup was compared to results obtained from pre-exposure baseline examinations as well as to data from a group of nonexposed control workers. There were no effects on hematology, clinical chemistry, ECG, pulmonary function, blood pressure, or heart rate 1 week after application.

The State of California requires that physicians report all cases of known or suspected pesticide exposures presented to them by patients. If a person experiences some pain/discomfort and merely suspects that they have been exposed to a pesticide, the case will be included as a "suspected illness" in the State's report. This liberal reporting procedure with no verification often results in the listing of a pesticide simply because the patient recalls using or being near the material at some point in the past and does not necessarily imply a cause-and-effect relationship. Based on this information, Pease *et al.* (1993) reported that glyphosate-containing products were the third most common cause of skin and eye irritation among agricultural workers and ranked 15th for systemic and respiratory symptoms. Relative to the level of product use, however, glyphosate ranked only 12th for the number of irritation symptoms reported.

Careful examination of the California data further indicates that the number of cases reported simply reflects greater use of the product relative to other herbicides and shows that glyphosate has relatively low toxicity among pesticides used in the State. Despite widespread use in California among pesticide

applicators and homeowners, there have been very few confirmed illnesses due to glyphosate (California EPA, 1996). In 1994, for example, glyphosate exposure was reported in only 25 cases, of which only 13 were considered "definitely or probably." Eleven of the 13 cases involved only minor and reversible eye irritation; the other two cases were a headache and an apparent misdiagnosis of reaction to hydrocarbon solvent, which is not an ingredient in Roundup. The California Department of Pesticide Regulation noted in its 1994 report that the majority of the people (c. 80%) affected by glyphosate experienced only irritant effects and, of the 515 pesticide-related hospitalizations recorded over the 13 years on file, none was attributed to glyphosate.

Acquavella *et al.* (1999) evaluated ocular effects in 1513 cases of Roundup herbicide exposure reported to a certified regional center of the American Association of Poison Control Centers (AAPCC) from 1993 through 1997. The large majority of reported exposures were judged by specialists at the center to result in either no injury (21%) or only transient minor symptoms (70%). None of the reported exposures resulted in permanent change to the structure or function of the eye. Based on these findings, it is concluded that the potential for severe ocular effects in users of Roundup herbicides is extremely low.

A limited number of studies have also investigated the results of occupational exposure in humans. Temple and Smith (1992) reported that accidental exposure to Roundup herbicide can result in eye and skin irritation. These investigators also reported other symptoms such as tachycardia, elevated blood pressure, nausea, and vomiting. However, such effects probably represent a nonspecific response related to the pain associated with eye and/or skin irritation. Talbot *et al.* (1991) found that accidental dermal exposure to six subjects did not result in any symptoms. Jamison *et al.* (1986) evaluated pulmonary function in workers handling flax which was previously retted (a process which softens and separates fibers by partial rotting) either by a dew-retting process or via the application of Roundup 6 weeks prior to harvest. It was reported that changes in pulmonary function were greater in the individuals exposed to preharvest retted flax compared to those inhaling the dew-retted vegetation. However, the levels of glyphosate still present in the flax which was sprayed 6 weeks before harvesting would be extremely low, if present at all, and could not be responsible for the altered pulmonary function observed. Rather, it is most likely that the two retting procedures produced dust particles with different physical characteristics and/or resulted in different microorganism populations in the retted vegetation.

Ingestion

Various studies reported in the literature describe the effects observed after accidental and intentional ingestion of Roundup. Accidental exposure results in, at most, only mild effects; no deaths have been reported. However, intentional ingestion of large amounts in suicide attempts has produced severe effects including severe hypotension, renal failure, and, in some instances, death (Sawada *et al.*, 1988; Menkes *et al.*, 1991; Talbot *et al.*, 1991; Tominack *et al.*, 1991; Temple and Smith, 1992). In those cases that result in mortality, death usually occurs within a few days of ingestion. In one study, it was estimated that the amount of concentrated Roundup intentionally ingested in fatal cases was 184 mL (range of 85 to 200), although it was noted that ingestion of much larger amounts resulted in only mild to moderate symptoms (Talbot *et al.*, 1991). Sawada *et al.* (1988) and Tominack *et al.* (1991) reported that average ingestion of 104 and 120 mL were not fatal while mean ingestion of 206 and 263 mL did produce death. Based on this information, it is concluded that the acute toxicity of Roundup in humans is low and is consistent with that predicted by the results of acute toxicity studies in rats.

The nature of the clinical symptoms observed in cases of suicide suggests that hypovolemic shock was the cause of death (Sawada *et al.*, 1988; Tominack *et al.*, 1989). Because similar responses have been observed in cases involving ingestion of other surface-active agents, it has been suggested that the acute toxicity of Roundup is likely due to the surfactant. This hypothesis is supported by results from a study in dogs that showed that the surfactant (POEA) produced a hypotensive effect, but glyphosate did not (Tai *et al.*, 1990). Based on other data, these investigators concluded that the hypovolemic shock was due to a cardiac depressant effect of very high doses of the surfactant. Talbot *et al.* (1991) reported that the clinical data generated in cases of intentional ingestion did not support hypovolemia as the cause of cardiovascular shock. Other factors, such as injury to the larynx and aspiration of vomitus into the lungs, were linked to mortality and specific pathological changes observed after intoxication with Roundup herbicide (Menkes *et al.*, 1991; Chang *et al.*, 1995; Hung *et al.*, 1997).

Summary

Results from several investigations establish that the acute toxicity and irritation potential of Roundup herbicide in humans is low. Specifically, results from controlled studies with Roundup showed that skin irritation was similar to that of a baby shampoo and lower than that observed with a dishwashing detergent and an all-purpose cleaner; no dermal sensitization, photoirritation, or photosensitization reactions were

observed. Furthermore, the incidence of occupational-related cases involving Roundup is low given the widespread use of the product. Data from these cases indicated some potential for eye and skin irritation with the concentrated product, but exposure to dilute spray solutions rarely resulted in any significant adverse effect. Most importantly, no lasting dermal or ocular effects were noted, and significant systemic effects attributable to contact with Roundup did not occur. Studies of Roundup ingestion showed that death and other serious effects occurred only when large amounts were intentionally ingested for the purpose of committing suicide. These data confirmed that the acute oral toxicity in humans is low and consistent with that predicted by the results of laboratory studies in animals.

EXPOSURE ASSESSMENT

Overview and Summary

Exposure assessment is generally conducted in a tiered manner, beginning with an assessment that employs simplifying assumptions to arrive at an upper bound estimate. When that upper limit exposure level is found to provide an adequate safety margin over toxicologic findings of concern, further refinement to identify a more accurate realistic exposure level is not generally undertaken. In the majority of instances, the first tier upper limit assessment overestimates actual exposure by 1 to 2 orders of magnitude.

Exposure of the general population to the components of Roundup herbicide is very low and occurs almost exclusively from the diet. Two population subgroups with maximal opportunity for additional exposure can be identified for purposes of this exposure assessment. These include professional pesticide applicators and children age 1 to 6 years. An upper limit on the magnitude of potential exposure to glyphosate, AMPA, and the POEA surfactant was calculated for these applicator and child subgroups, based on the sum of highest possible exposures by dietary and other possible exposure routes. Realistic exposure for these subgroups and for the general population is expected to be a small fraction of this extreme estimate.

Applicators are directly involved during herbicide spraying operations and can be exposed on a repeated basis. Although this exposure through occupational activities does not necessarily occur each day for a working lifetime, herbicide exposure was treated as chronic to establish an upper bound estimate. To be conservative, the applicator's body weight was assumed to be 65.4 kg, in order to account for both male and female workers. This approach was designed to provide a maximum estimate of exposure on a milligrams per kilogram of body weight per day basis. Children age 1 to 6 years experience the highest dietary exposure because they eat more food per kilogram of body weight than

other age groups. Young farm children may also contact pesticide residues in their surrounding environment and thus have more opportunity for potential incremental exposure. We therefore selected this age class as a high-end subgroup for nonoccupational exposure among the general population.

Worst-case estimates of exposure to glyphosate, AMPA, and POEA were calculated for aggregated acute and chronic exposure scenarios. The aggregate exposure for chronic scenario was based on the ingestion of food commodities and drinking water containing trace residues in addition to exposures from the spraying of Roundup by applicators. The acute scenario incorporated occasional, inadvertent exposure routes (spray drifting onto bystanders, reentry into previously treated areas). This scenario also included additional sources from unintentional exposures that can occur on a rare basis during specific activities (e.g., consumption of wild berries and mushrooms that might be sprayed inadvertently; the activity of swimming in a pond with herbicide residues). The aggregated acute scenario included the chronic exposure sources in addition to exposure resulting from these inadvertent exposure routes.

Though worst-case assumptions were used throughout, the calculated exposures to glyphosate, AMPA, and POEA were shown to be low (Table 5). Calculating for glyphosate, acute and chronic exposures to applicators were 0.125 and 0.0323 mg/kg body wt/day, respectively; for young children, the values were 0.097 and 0.052 mg/kg body wt/day. Estimates of exposure to AMPA were also very low, ranging from 0.0048 to 0.0104 mg/kg body wt/day. The calculated exposures for POEA ranged from 0.026 mg/kg body wt/day for chronic exposure in children to 0.163 mg/kg body wt/day for acute applicator exposure.

Conservative assumptions used in analysis of both the acute and the chronic exposure scenarios ensure that conditions for upper-limit or worst-case exposure estimates were established. For example, estimates of dietary intake used maximum residue levels (MRLs), the highest legal residue levels allowed on crops. If actual measured residue levels were used in place of the MRL values and other factors were considered (e.g., percentage of crop treated, reduction in residues from washing, processing), dietary exposure estimates would be substantially reduced (10- to 100-fold or more). Estimates of acute drinking water exposure used the highest measured value resulting from 5 years of drinking water monitoring in the United Kingdom (1.7 ppb). This conservative assumption exaggerates glyphosate exposure, since 99% of the UK data did not detect glyphosate above 0.1 mg/L. For applicators, the highest measured value from all monitoring work was used to estimate acute exposures. Conservative estimates were included for other sources of exposure as well. Exposure estimates using more realistic as-

TABLE 5
Worst-Case Daily Exposure Estimates for Glyphosate, AMPA, and POEA (mg/kg/day)

Nature/source of exposure	Glyphosate				AMPA				POEA			
	Female adult applicator		1- to 6-year-old female child		Female adult applicator		1- to 6-year-old female child		Female adult applicator		1- to 6-year-old female child	
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Routine												
Dietary	23.8	23.8	51.9	51.9	4.8	4.8	10.4	10.4	11.9	11.9	26	26
Application	56.2	8.5	0	0	0	0	0	0	128	20.6	0	0
Occasional												
Drinking water	3.6 $\times 10^{22}$	2.1 $\times 10^{23}$	0.11	6.7 $\times 10^{23}$	2.1 $\times 10^{23}$	2.1 $\times 10^{23}$	6.7 $\times 10^{23}$	6.7 $\times 10^{23}$	1.8 $\times 10^{22}$	1.1 $\times 10^{23}$	5.5 $\times 10^{22}$	3.3 $\times 10^{23}$
Reentry	0	0	26	0	0	0	0	0	0	0	65.0	0
Bystander	0	0	4.4	0	0	0	0	0	0	0	9.8	0
Infrequent/rare												
Swimming	1.28	0	6.5	0	0	0	0	0	0.64	0	3.2	0
Wild Foods	45	0	45	0	0	0	0	0	23	0	23	0
Aggregate ^a	125	32.3	97	52	4.8	4.8	10.4	10.4	162.9	32.5	91.1	26

^a Aggregate exposure is the sum of dietary, drinking water, and application derived contributions, plus 45 mg glyphosate/kg/day or either 23 (adults) or 65 (children) mg POEA/kg/day acute exposure to account for all incidental exposures related to occasional behaviors. For AMPA, aggregate exposure is the sum of dietary and drinking water contributions, since no other routes provided significant incremental contributions.

sumptions than those described in Table 9 would yield substantially lower values than those determined in this assessment, and thus the worst-case analysis exposure estimates represent overestimates.

Dietary Exposure to Residues in Food

Glyphosate

In order to obtain approval for the application Roundup onto food or feed crops, it is necessary to measure residues of herbicide and related products that represent the maximum levels of glyphosate and AMPA that hypothetically occur in food using the highest and most frequent herbicide applications. These data support legally binding MRLs (called "tolerances" in the United States) that are established in most countries worldwide for the resulting food commodities. In addition, international MRLs continue to be established by Codex Committee on Pesticide Residues to facilitate international trade of agricultural products.

An initial benchmark for assessment of maximum dietary exposure can be obtained by making the simplifying assumption that all food commodities contain the highest legal residue levels (MRLs). This calculation relies on the unrealistic assumptions that 100% of crop acreage is treated with Roundup at the highest allowed rates and that all resulting food contains the greatest permissible residues, which are not reduced through processing, washing, or cooking. When glyphosate MRLs are multiplied by average daily food consumption data and summed for all foods that can be treated, a theoretical maximum daily intake (TMDI) exposure is calculated. Of course, there are differences among countries in the magnitude of established MRLs and in food consumption estimates. The WHO considers 86 regional diets in the Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) when making safety assessments for Codex MRLs (WHO, 1997). Comparison of present MRLs among different countries indicates that U.S. MRLs for glyphosate are both more numerous and of equal or greater magnitude than in most other countries. The resulting U.S. TMDI should therefore represent an upper bound exposure compared to other jurisdictions.

The TAS EXPOSURE-1 software⁵ incorporates food consumption data for all U.S. crop commodities and provides a dietary exposure estimate for the U.S. population as a whole and for more than 20 specific population subgroups. Using the present U.S. MRLs, the TAS model provided TMDI exposure estimates for

⁵ Technical Assessment Systems, Inc. (TAS). Exposure-1 software. TAS, Inc. The Flour Mill, 1000 Potomac St. NW, Washington, DC 20007. 1-202-337-2625. Calculations completed using 1977-1978 food consumption data.

glyphosate residues of 23.8 mg/kg body wt/day for the U.S. population and 51.9 mg/kg body wt/day for children age 1 to 6 years. These values represent maximum daily dietary exposure for the adult worker and the child subgroups, respectively, for both the chronic and the acute scenarios. These glyphosate exposure estimates include contributions from all presently allowed uses, including all currently approved glyphosate-tolerant crops. These dietary exposure estimates are slightly higher than comparable estimates obtained from the WHO dietary consumption model or the German intake model (Kidwell *et al.*, 1995) because of regional differences in food consumption and MRLs. Refinement of this maximum estimate could be achieved from a consideration of actual measured residue levels rather than MRLs, realistic application rates, the fraction of crops actually treated, and the effect of processing, washing, cooking, blending, etc. Thus, actual values could be incorporated to arrive at more realistic exposures. For example, U.S. residue data from wheat treated with maximum rates of Roundup showed the highest glyphosate residue to be 2.95 mg/g, with a mean level of 0.69 mg/g, compared to a MRL of 5 mg/g (Allin, 1989). Glyphosate-tolerant soybeans treated at maximum allowed rates and frequency contained glyphosate residues at the highest level of 5.47 mg/g, with a mean of 2.36 mg/g, compared to the MRL of 20 mg/g (Steinmetz and Goure, 1994). Clearly, only a fraction of cropped acres receive a Roundup treatment, which can be estimated to be in the range of 10 to 50%. Because the ingredients in Roundup are water soluble, processing, washing, and cooking are expected to further reduce residues. Therefore, considering the combination of factors, it is expected that realistic chronic dietary exposure to glyphosate and the other ingredients in Roundup are at least 1 to 2 orders of magnitude lower than the TMDI estimates used in this assessment. Greater accuracy in these refinements is not needed at this time for glyphosate, because even the extremely conservative TMDI assessments have shown that dietary exposure are acceptable compared to dosages leading to experimental toxicological findings (see Table 9).

AMPA

AMPA has historically been considered a minor part of the plant residue derived from glyphosate treatment. Measured levels of AMPA in plant residue studies have averaged about 10% of the glyphosate level (U.S. EPA, 1993) and have been summed with glyphosate to arrive at total residue for MRL setting and risk assessment purposes (U.S. EPA, 1997b). Some jurisdictions have determined that AMPA is not of toxicological concern (U.S. EPA, 1993) and do not include it in MRLs any longer. Canada and the JMPR have proposed to establish a separate MRL for AMPA in cases

where it is the major residue in glyphosate-tolerant crops that express an enzyme that converts glyphosate to AMPA as a mechanism of tolerance.

In order to arrive at a maximum estimate of AMPA dietary exposure, it has been assumed that AMPA represents 20% of the TMDI glyphosate exposure. This is a compromise between the bulk of the historical data that indicates that AMPA residues are 10% of glyphosate levels and the more recent findings that specific glyphosate-tolerant crops have a higher ratio. Based on this assumption, AMPA dietary exposure was 4.8 mg/kg body wt/day for the U.S. population and 10.4 mg/kg/day for children age 1 to 6 years.

POEA

Dietary exposure to POEA surfactant is not significant, since surfactants are not believed to be systemically transported in crop plants in the same manner as glyphosate and AMPA (Sherrick *et al.*, 1986; Smith and Foy, 1966). The assumption made for purposes of this assessment was that residues would occur in proportion to glyphosate exposures, based on the relative amount of each in the formulation (2:1, glyphosate: POEA). Using this ratio, TMDI exposure for POEA residues are 11.9 and 26 mg/kg body wt/day for the U.S. population and for children age 1 to 6 years, respectively.

Occupational Dermal and Inhalation Exposure during Application

The level of worker exposure to Roundup during herbicide spraying applications has been reported in both forestry (Centre de Toxicologie du Quebec, 1988; Jauhainen *et al.*, 1991; Lavy *et al.*, 1992) and agricultural (Kramer, 1978) sites. Most studies have used passive dosimetry to determine the quantity of herbicide deposited during spraying. Deposition is measured from analysis of material from gauze patches located on workers skin and clothing. These deposition results provide a basis for calculating systemic exposure using *in vivo* data for dermal penetration of glyphosate that shows 2% or less reaches systemic circulation (Wester *et al.*, 1991). Inhalation exposure was determined by measurement of glyphosate levels in air sampled from the workers' breathing zones. This allowed calculation of exposure estimates using hourly breathing rates (U.S. EPA, 1997a) and making the further assumption that all inhaled spray mist was bioavailable. Some studies have also utilized urine monitoring of exposed workers to quantify excreted glyphosate (Lavy *et al.*, 1992). Workers' body burdens were calculated based on data showing that .95% of glyphosate administered intravenously to rhesus monkeys is excreted via urine (Wester *et al.*, 1991).

In field studies used to estimate exposure, workers generally wore protective clothing as directed accord-

ing to the label, and that was considered normal for their occupation. They performed a variety of duties, including mixing and loading spray solutions, backpack, handgun, and boom spraying, weeding, and scouting fields. In the studies utilizing passive dosimetry, gauze patches from both outside and inside of shirts were analyzed to determine the degree of protection provided by work clothing.

Taken together, these studies show that dermal and inhalation exposure to Roundup during application is very low. Body burden doses of glyphosate resulting from dermal contact during application measured by passive dosimetry methods ranged from 0.003 to 4.7 mg/kg body wt/work h. Clothing reduced exposure to the arms an average of 77% (Lavy *et al.*, 1992). Glyphosate levels in applicators' breathing air ranged from undetectable to 39 mg/m³ of air (Kramer, 1978), with the vast majority of quantifiable results being less than 1.3 mg/m³ (Jauhiainen *et al.*, 1991). Tank-filling operations created the highest dermal exposure (hands), ranging from 4–3 × 10²² to 12 mg/kg body wt/filling operation (Kramer, 1978), assuming that each operation lasted 10 min.

The results of biological monitoring showed that most of 350 urine samples analyzed from workers contained no measurable glyphosate, with detection limits ranging from 0.01 to 0.1 mg/mL. On a few isolated occasions, urine levels of 0.025 to 0.095 mg/mL were found, although urine volume data were not provided to permit accurate estimation of body burden (Centre de Toxicologie du Quebec, 1988; Jauhiainen *et al.*, 1991). The maximum body burden among workers based on urine monitoring data has been estimated at 8.0–3 × 10²² mg/kg body wt/h worked, assuming that all urine without measurable glyphosate contained concentrations of one-half of the method's detection limit (Lavy *et al.*, 1992). The monitoring estimate based on urine herbicide levels was within the range of passive dosimetry predictions, thus lending support to the utility of passive monitoring techniques as reasonable measures of true exposure.

For the present assessment of an adult applicator working for 8 h per day, weighing 65.4 kg and breathing 1.3 m³ of air/h during moderate outdoor exertion (U.S. EPA, 1997a), a maximum daily acute exposure to glyphosate was estimated using the highest of the above reported measurements. Dermal exposure from one 10-min mixing and loading operation was 12 mg/kg body wt. Dermal exposure was 38 mg/kg body wt, and inhalation exposure was 6.2 mg/kg body wt during 8 h of application. Summed together, the adult worker's peak acute exposure during application was calculated as 56.2 mg/kg body wt/day.

Chronic applicator exposure was estimated using average rather than peak exposure measurements. Average exposure during a 10-min tank-filling operation was 6.3 mg/kg body wt (Kramer, 1978). Average dermal

exposure (Kramer, 1978; Lavy *et al.*, 1992) during application was 5.1 mg/kg body wt/day. Average air concentration was difficult to calculate, since many measurements were below detection limits (Jauhiainen *et al.*, 1991). Utilizing an average air concentration of 2.87 mg/m³ from Kramer (1978), where the assumption was made that the air concentration associated with each undetectable result was at the detection limit, chronic inhalation exposures for the applicator were 0.46 mg/kg body wt/day. Summed together, and amortizing for a 5-day working week, chronic applicator exposure to glyphosate was estimated to be 8.5 mg/kg body wt/day.

AMPA

There is no application-related exposure to AMPA, since its presence is dependent on environmental degradation and therefore not present in spray solutions. However, calculations were made for predicting rat NOAELs based on AMPA in technical glyphosate.

POEA

No data were available that directly quantify systemic exposure to POEA arising from application. Dermal deposition or inhalation of POEA would occur in proportion to glyphosate exposures, based on the relative amount of each in the formulation, as above. It was further assumed that dermal penetration of POEA was 10% of that deposited on skin, which is a conventional default assumption for surfactants (Martin, 1990; Lundehn *et al.*, 1992). Based on these assumptions, utilizing the glyphosate exposure data, peak acute 1-day systemic exposure to POEA was calculated to be 30 mg/kg body wt (dermal during one mixing and mixing/loading operation), 95 mg/kg body wt (dermal during application), and 3.1 mg/kg body wt (inhalation). Summed, the total acute daily exposure was 128 mg/kg body wt. Chronically, using the same assumptions and amortizing for a 5-day work week, mixing/loading contributed 11.3 mg/kg body wt/day, dermal exposure during application contributed 9.1 mg/kg body wt/day, and inhalation contributed 0.23 mg/kg body wt/day. Summed, chronic application-related exposure to POEA was estimated to be 20.6 mg/kg body wt/day.

Nonoccupational Exposure during Application

Nonoccupational application-related acute exposures to Roundup can also occur during residential applications of Roundup to control problem weeds in the home and garden. These applications will be primarily spot treatments and edging, utilizing very small quantities on a few occasions during a year. Occupational exposure data, normalized to a kilogram of glyphosate applied basis, showed the highest exposure was 28 mg of glyphosate/kg body wt/kg of glyphosate

applied (Lavy *et al.*, 1992). It was acknowledged that homeowners may not be well trained in application techniques nor always utilize appropriate personal protective equipment. Therefore, the maximum residential exposure was estimated to be 10-fold greater than the highest measured for the forestry workers (up to 280 mg/kg body wt/kg applied). If a homeowner applied an entire 10-L container of Ready-To-Use Roundup spray solution (1% glyphosate concentration) and experienced such an exaggerated exposure, the summed inhalation and dermal exposure would be 28 mg/kg body wt or about 50% of the peak acute occupational exposure. Based on this analysis, the risk assessment for adult occupational application-related exposure is sufficient to cover nonoccupational homeowner exposures.

Consumption of Water

Glyphosate

Glyphosate has rarely been detected in drinking water, even though many studies have been done. This is expected because it binds tightly to soil and degrades completely into natural substances (U.S. EPA, 1993; WHO, 1994a). The maximum concentration of glyphosate in well water identified in the scientific literature was 45 mg/L, which was reported 21 days after the second application of Roundup at a very high rate (4.6 kg/ha) to a gravel soil surrounding an electrical substation in Newfoundland (Smith *et al.*, 1996). This was not a drinking water well, but it serves as an extreme worst-case upper limit for glyphosate measured under field conditions. As a result of the 0.1 mg/L limit for any pesticide in drinking water in the European Union, many thousands of drinking water samples have been routinely analyzed for glyphosate and other pesticides. The best available data on glyphosate levels in drinking water was obtained from the United Kingdom Drinking Water Inspectorate. During the years 1991 to 1996, 5290 samples derived from surface and ground water sources were analyzed (Hydes *et al.*, 1996, 1997). All but 10 were below the 0.1 mg/L limit. Among those 10 reported detections, concentrations ranged from 0.2 to 1.7 mg/L. The exceedences detected have not been confirmed by follow-up investigation, and it is possible that some are false positives, since follow-up investigation of other low-level positive water detections have often not confirmed the initial report. As an example, 1 of the 10 UK detections was a sample from Llanthony, Wales, that was initially reported to have 0.53 mg glyphosate/L. Subsequent investigation of the site and repeated sampling and analysis did not reveal any amount of glyphosate in the water supply, nor could the source of the initial false finding be identified (Palmer and Holman, 1997). Even allowing for the assumption that all 10 UK detections are accurate,

99th percentile exposure to glyphosate via drinking water is below 0.1 mg/L.

Irrespective of measured concentrations, U.S. EPA has established a maximum contaminant level (MCL) of 700 mg/L as a health-based upper legal limit for glyphosate in drinking water (U.S. EPA, 1992b). However, using the GENECC and SCI-GROW environmental fate models, U.S. EPA more recently estimated glyphosate concentration in drinking water for the purpose of risk assessment (U.S. EPA, 1998). These fate models were used by the U.S. EPA as coarse screening tools to provide an initial sorting of chemicals with regard to drinking water risk. U.S. EPA concluded from the models that the average concentrations of glyphosate that could be expected in surface and ground water, respectively, were 0.063 and 0.0011 mg/L, 4 to 5 orders of magnitude below the MCL that is legally considered safe for chronic exposure.

Surface waters can be directly treated with Roundup for the purpose of aquatic weed control, which can lead to temporary glyphosate levels in water. However, it is believed that all surface waters that would subsequently be used for drinking purposes would undergo various purifying treatments, such as standard chlorine or ozone treatments. These treatments are known to be effective at removing glyphosate and AMPA from the water (Speth, 1993).

It is difficult to identify appropriate upper-limit glyphosate concentrations that can be used to characterize acute and chronic exposure from drinking water. If regulatory limits are selected, predicted exposure could vary through many orders of magnitude, depending on the jurisdictional limits used. Therefore, for this assessment, the peak acute exposure was considered to be no more than 1.7 mg/L, the highest reported measured value in the UK drinking water program. The same data indicated that chronic exposure could not exceed 0.1 mg/L, the European Union exposure limit. This value is supported by the U.S. EPA model calculations. Based on figures for mean daily water consumption and body weights (U.S. EPA, 1997a) for an adult (1.4 L and 65.4 kg) and a preschool child (0.87 L and 13 kg), the acute exposure to glyphosate from drinking water was calculated to be 3.6×10^{-22} (adult) and 0.11 (child) mg/kg body wt. The chronic exposures, calculated in the same manner, were 2.1×10^{-23} (adult) and 6.7×10^{-23} (child) mg/kg body wt/day.

AMPA

AMPA can also occur in water as a result of glyphosate degradation following Roundup treatments, although its peak concentration is found later and at levels that are only 1 to 3% of peak glyphosate concentrations (Feng *et al.*, 1990; Goldsborough and Beck, 1989). To be conservative and still consistent with the glyphosate assessment above, AMPA levels were as-

sumed to be 0.1 mg/L for both the acute and the chronic exposure levels. Calculations using the body weight and consumption parameters described predicted acute and chronic adult and child exposures as 2.1×10^{23} and 6.7×10^{23} mg/kg body wt/day, respectively. These water-derived AMPA exposures are much less than 1% of those derived from food and are therefore essentially insignificant, eliminating a need for further refinement of the concentration information. AMPA can also be formed from degradation of phosphonate detergents and sequestering agents used in cooling water treatment (Steber and Wierich, 1987), but possible exposures derived from nonglyphosate sources were not considered here.

POEA

No direct analytical data were found from which exposures to POEA via drinking water could be independently estimated. Surfactants are expected to bind tightly to soil and sediment particles and dissipate quickly via microbial degradation (Van Ginkel *et al.*, 1993; Giger *et al.*, 1987). For the present assessment, the level of POEA in drinking water was assumed to be proportionate to glyphosate exposures, based on the relative amount of each in the formulation, as discussed above. Acute exposure to POEA from drinking water was calculated to be 1.8×10^{22} (adult) and 5.5×10^{22} (child) mg/kg body wt. The chronic exposures, calculated in the same manner, were 1.1×10^{23} (adult) and 3.3×10^{23} (child) mg/kg body wt/day.

Reentry of Treated Areas

Glyphosate

Exposure to glyphosate during worker reentry into agricultural fields 1, 3, and 7 days after Roundup treatment has been measured using the passive dosimetry methods (Kramer, 1978). Two fields studied contained a mixed population of 0.5 m tall grasses and very tall (1.5 m) grassy weeds, while one was composed only of the shorter weeds. As expected, inhalation exposure during reentry was negligible because spray mist had dissipated and glyphosate is a nonvolatile salt (Franz *et al.*, 1997). Based on the measured 2% dermal penetration rate (Wester *et al.*, 1991) acute exposures derived from these data were 3.9×10^{23} to 2.6×10^{23} mg/kg body wt/h for an adult, with a mean value of 0.52 mg/kg body wt/h. Exposures were 10-fold greater for reentry into tall grass compared to short, and potential for exposure decreased over time posttreatment, with values on day 7 averaging 3% of those on day 1. Adjusting for a child's body surface area of 40% that of an adult (Richardson, 1997; U.S. EPA, 1997a) and a child's lower body weight, exposures of a child reentering the same fields were calculated to be 0.01 to 5.2 mg/kg body wt/h.

One scenario to consider assumes that a 1- to 6-year-

old farm child could on occasion enter a recently treated field and could remain there either playing or helping a parent for a significant period of time. Such activity might occasionally occur for a 5-h period on a particular day, producing a maximum exposure of 26 mg of glyphosate/kg body wt for the child. This route of exposure for a child was considered to be an infrequent, acute event with no calculation necessary to account for chronic exposure.

The calculations above indicated that maximum female adult dermal reentry exposure rate to glyphosate on an hourly basis was 55% of peak dermal exposures experienced during application activities, and the ranges were of similar magnitude. Since acute and chronic applicator exposure levels have been established for the worker, these values, therefore, also account for any reentry exposure a woman may experience as part of her other activities. During any work time period, a woman can be making an application or reentering a recently treated field, but not both, since Roundup's herbicidal effects develop too slowly to justify repeated treatment after periods of less than 2 weeks.

AMPA

Since reentry exposure involves transfer from treated surfaces, no AMPA would be present, because AMPA is produced by metabolic conversion in a plant or within soil microbes and would not be found as surface residue.

POEA

POEA surfactant would be deposited on surfaces in a ratio that is proportional to its concentration in the formulation and would therefore be available from surface contact. Acute exposure was calculated to be 65 mg/kg body wt for the child, after adjusting for the assumed greater (10%) dermal penetration rate. Reentry exposures to POEA for the adult worker would be less than experienced by an applicator and should be covered by the applicator-derived exposure assessment.

Bystander Exposure during Application

It is also possible for the farm child bystander to experience inadvertent acute dermal and inhalation exposure to Roundup from spray drift during an application, if he/she is adjacent to the application area. Substantial scientific research has been devoted to measurement, estimation, and modeling of off-site spray drift (Grover, 1991). The expected exposure is a fraction of the target treatment rate, reduced by a factor influenced by the separation distance, environmental variables, and application parameters. Aerial applications maximize drift because the droplets are

released at a higher altitude. For preliminary ecological risk assessment, U.S. EPA has assumed spray drift exposures could be 5% of the aerial application rate (U.S. EPA, 1995). Off-target deposition of glyphosate has been measured (Feng *et al.*, 1990), and after aerial application, less than 0.1% of the on-site deposition was intercepted 8 m from the spray boundary.

For the purpose of retaining maximum conservatism, it was assumed that off-site bystander dermal and inhalation exposures could be 10% of an applicator's on-site peak 8-h acute exposures (calculated above). Contributions from mixing and loading operations were excluded. The summed calculated exposure estimate for the child bystander was 4.4 mg of glyphosate/kg body wt/day. No adjustment was made for the child's reduced breathing volume, body weight, or skin surface area, because this was intended as a simple upper bound estimate. No application-related bystander exposure to AMPA will occur, since it is only formed upon environmental degradation. Daily POEA acute exposure, based on relative concentrations in the formulation and calculated as 10% of peak on-site applicator exposure, was 9.8 mg/kg body wt. Such bystander exposures would be infrequent, since Roundup is only applied to a given location a few times each year, at most, and were considered only for the acute risk scenario.

Possible Inadvertent Exposures Derived from Specific Activities

In the course of this assessment, preliminary estimates were made to determine whether other possible inadvertent environmental contact might contribute significantly to incremental glyphosate exposures. Several routes of exposure were considered for glyphosate, AMPA, and POEA. These included (1) dermal contact with or accidental ingestion of treated soil; (2) inhalation or ingestion of residential dust derived from treated soil; (3) dermal contact with waters or aquatic sediments during swimming or showering; (4) accidental ingestion of treated surface waters while swimming; and (5) ingestion of inadvertently sprayed wild foods such as berries or mushrooms. Using standard exposure parameters (U.S. EPA, 1988, 1992b, 1997a) and conservative assumptions about expected environmental concentrations and frequency of such contact, only the latter two potential incremental exposure routes were found to contribute possible exposures greater than 1 mg/kg body wt/day. Infrequent incremental exposures below this level were judged to be insignificant compared to recurring dietary, drinking water, and application-related exposure levels.

Glyphosate formulations can be used to control surface weeds on ponds, lakes, rivers, canals, etc., according to label rates up to about 4.2 kg glyphosate per hectare, which can result in significant water concen-

trations immediately after treatment. These glyphosate levels in water dissipate quickly (Goldsborough and Beck, 1989), and it is unlikely that such weedy water bodies would attract swimmers or bathers. However, if such an application were made to water 0.25 m deep, the immediate resulting glyphosate concentration could be 1.68 mg/mL if it were mixed into the water column. It has been estimated that accidental ingestion of water during 1 h of swimming could be 50 mL (U.S. EPA, 1988), so maximal incremental exposure to glyphosate was estimated to be 1.28 and 6.5 mg/kg body wt for a swimming adult and child, respectively. Such exposures will be very rare and therefore only were considered as a possible increment to the acute exposure scenario. AMPA will not be present at significant concentrations in water shortly after treatment. POEA surfactants are not necessarily included in glyphosate formulations intended for aquatic uses. If a surfactant were to be included in an application to aquatic systems, such a substance would be applied at doses approximately half that of glyphosate. We conclude that swimming in water from areas recently treated with Roundup would produce an incremental POEA oral exposure potential of 0.64 and 3.2 mg/kg body wt for a swimming adult and child, respectively.

Roundup application along roadsides or in forestry creates the potential for accidental overspray of wild foods that could later be collected for consumption. Consideration of actual use patterns, the percentage of forests or roadsides that actually receive treatment, and the resulting phytotoxic effects on the sprayed plants suggests that inadvertent exposure will be extremely unlikely. However, since residue levels of glyphosate arising from a mock overspray of berries has been measured (Roy *et al.*, 1989), the potential dietary exposure was quantified. Peak glyphosate residue levels in raspberries were 19.5 mg/g (Roy *et al.*, 1989), and it was estimated that maximal consumption for an individual might be 150 g for an adult and 30 g for a 1- to 6-year-old child. These parameters predict an exposure of 45 mg/kg body wt for both subgroups and relies on the assumption that the surface residues were not reduced by washing before consumption. Exposure at this level is approximately equal to the total TMDI dietary estimate, suggesting that it could be a significant but rare incremental contributor to acute exposure scenario. AMPA residues were also quantified in the raspberries, but were less than 1% of those for glyphosate (Roy *et al.*, 1989) and are therefore insignificant. POEA surfactant residues were not measured, but can be assumed to be 50% of those for glyphosate, based on the relative formulation content, leading to potential incremental oral POEA exposures of 23 mg/kg.

Aggregate Exposure Estimates

The calculated acute and chronic exposure estimates for each population subgroup for glyphosate, AMPA, and POEA are summarized in Table 5. For glyphosate, acute exposures to applicators and children were calculated to be 0.125 and 0.097 mg/kg body wt/day, respectively; chronic exposures in these subgroups were 0.0323 and 0.052 mg/kg body wt/day, respectively. Levels of exposure to AMPA were very low (0.005 ± 0.010 mg/kg body wt/day). Estimates of exposure to POEA were 0.163 and 0.0911 mg/kg body wt/day for the acute scenarios, while chronic exposure estimates were four to five times lower than the acute values.

RISK CHARACTERIZATION

Introduction

Risk characterization involves a determination of the likelihood that an adverse health effect will result from exposure to a given substance. The method used in this assessment to characterize risk was the margin of exposure (MOE) analysis, in which dose levels from animal toxicity tests were compared to conservative, upper-limit estimates of human exposure. To evaluate the risks resulting from chronic exposure, estimates of human exposure were compared to the lowest dose that produced no adverse effects in repeat dose studies with animals. For acute effects, human exposure estimates were compared to oral LD₅₀ values in rats. The MOE is defined as the quotient of the NOAEL divided by the aggregate human exposure calculated from total daily intake from all sources.

The introduction of safety factors is a concept that has had wide acceptance in the scientific and regulatory communities around the world. The Joint European Committee on Food Additives (JECFA) proposed principles for determining a margin of safety (MOS) and has developed a methodology to establish an acceptable value for a factor that would directly link animal toxicological data to human health and safety (FAO/WHO, 1958). For purposes of extrapolation of data from animals to man, the figure is based on an established dosage level that causes no demonstrable effects in the animals. The MOS allows for any species differences in susceptibility, the numerical differences between the test animals and the exposed human population, the greater variety of complicating disease processes in the human population, the difficulty of estimating the human intake, and the possibility of synergistic action. JECFA stated that the 100-fold margin of safety applied to the maximum ineffective dosage (expressed in mg/kg body wt/day) was believed to be an adequate factor (FAO/WHO, 1958). The value of 100 has been regarded as comprising two factors of

ten to allow for interspecies and interindividual (intraspecies) variation (WHO, 1994b).

The validity and size of safety/uncertainty factors and their application across many substances including pesticides have undergone periodic reevaluation (Renwick and Lazarus, 1998). By and large the allocation of appropriate safety factors is considered on a case-by-case basis, relying on analysis of the total weight of evidence including a consideration of data gaps (WHO, 1990). WHO Scientific Groups have confirmed a 100-fold safety factor as an adequate and useful guide, particularly when there are few toxicological data gaps (WHO, 1967, 1994b).

The National Research Council Report on Pesticides in the Diets of Infants and Children (NRC, 1993) indicated that the current 10-fold intraspecies factor adequately protects for socioeconomic, nutritional, and health status factors that influence the vulnerability of children to environmental toxicants. The NRC report (NRC, 1993) also indicated the possible requirement for an additional 10-fold uncertainty factor to be applied to the ADI for pesticide residues in food to protect infants in the absence of specific data on developmental toxicity. The Environmental Protection Agency sometimes applies a 3- to 10-fold margin of safety for infants and children in the case of threshold effects. This additional factor would account for pre- and post-natal toxicity and is applied when existing data indicate a possible increased sensitivity to infants or to children or when the database of effects is incomplete (U.S. EPA, 1998a).

Recently the U.S. EPA conducted a review of the risks associated with aggregate exposures to glyphosate residues from all sources (U.S. EPA, 1998a). Using a margin of exposure analysis, it was concluded that reliable data support the use of the standard 100-fold uncertainty factor for glyphosate, and that an additional ten-fold uncertainty factor is not needed to protect the safety of infants and children.⁹ There was no suggestion of increased severity of effect in infants or children or of increased potency or unusual toxic properties of glyphosate in infants and children. Therefore, in the view of U.S. EPA, there are no concerns regarding the adequacy of the standard MOE/safety factor of 100-fold (U.S. EPA, 1998a).

Identification of NOAELs

The toxicity of glyphosate and AMPA has been investigated in a comprehensive battery of studies. In addition, POEA has been tested in acute, subchronic, genetic, and developmental toxicity studies. A summary of the no-effect levels identified in the various studies conducted with these materials is provided below and in Tables 6-8. The no-effect levels selected for risk characterization are discussed below.

TABLE 6
Glyphosate NOAELs for Toxicological Endpoints

Type of study and species tested	NOAEL (mg/kg/day)	Comments	Study reference
Subchronic toxicity			
Mouse, 90-day	2310	Based on decreased b.w. ^a gain	Tierney, 1979
Mouse, 90-day	630	Based on salivary gland lesions	NTP, 1992
Rat, 90-day	\$ 1445	No adverse effects at HDT ^b	Stout, 1987
Rat, 90-day	209	Salivary gland changes at the lowest dose tested not considered toxicologically significant	NTP, 1992
Dog, 12-month	\$ 500	No adverse effects at HDT	Reyna and Ruecker, 1985
Chronic toxicity			
Mouse, 24-month	885	Based on liver effects	Knezevich, 1983
Rat, 26-month	\$33	No adverse effects at HDT	Lankas, 1981
Rat, 24-month	409	Based on decreased b.w. gain and ocular lesion	Stout and Ruecker, 1990
Developmental toxicity			
Rat	1000	Based on maternal and fetal effects	Tasker, 1980a
Rabbit	175	Based on maternal toxicity	Tasker, 1980b
Reproductive toxicity			
Rat	\$30	No adverse effects at HDT	Schroeder, 1981
Rat	694	Based on systemic toxicity; no reproductive effect	Reyna, 1990

^a b.w., body weight.

^b HDT, highest dose tested.

Glyphosate

The lowest no-effect level for purposes of risk characterization for adults is the NOAEL of 175 mg/kg body wt/day; this value is based on the occurrence of maternal toxicity at the highest dosage tested (350 mg/kg body wt/day) in the rabbit developmental toxicity study. The NOAELs in the chronic rodent or dog stud-

ies, multigeneration reproduction studies and the rat developmental toxicity study ranged from approximately 400 to 1000 mg/kg body wt/day.

Calculation of an MOE based on the endpoint of maternal toxicity is biologically irrelevant for the young (1 to 6 years). Nevertheless, such an analysis was conducted by the U.S. EPA and is included here to

TABLE 7
AMPA NOAELs for Toxicological Endpoints

Type of study and species tested	NOAEL (mg/kg/day)	Comments	Study reference
Subchronic toxicity			
Rat, 90-day	400	Based on urinary tract infection	Estes, 1979
Dog, 90-day	263	No adverse effects at HDT	Tompkins, 1991
Chronic toxicity	. 2.8	AMPA present at 0.68% in glyphosate study; no effects at middose	Stout and Ruecker, 1990
Rat, 24 month			
Developmental toxicity			
Rat	400	Based on maternal and fetal b.w. ^a effects	Holson, 1991
Reproductive toxicity			
Rat	. 4.2	AMPA present at 0.61% in glyphosate study; no effects at middose	Reyna, 1990

^a b.w., body weight.

TABLE 8
POEA NOAELs for Toxicological Endpoints

Type of study and species tested	NOAEL (mg/kg/day)	Comments	Study reference
Subchronic toxicity			
Rat, 1-month	57	Based on decreased b.w. ^a gains	Ogrowsky, 1989
Rat, 3-month	36	Based on decreased b.w. and intestinal irritation	Stout, 1990
Dog, 14-week	30	Based on reduced b.w. and gastrointestinal irritation	Filmore, 1973
Developmental toxicity			
Rat	15	Based on slight decrease in food consumption and mild clinical signs	Holson, 1990

^a b.w., body weight.

demonstrate that even use of an unrealistic assumption provides an acceptable margin of exposure. The NOAEL of 209 mg/kg body wt/day from the second subchronic rat study (NTP, 1992) was also used to calculate the MOE for children because this value was the next higher no-effect level and was based on a more relevant toxicological endpoint.

AMPA

Some regulatory agencies have determined that AMPA is not of toxicological concern and do not include it in assessments of risk. Other agencies have summed AMPA with glyphosate to arrive at total exposure for risk assessment purposes. Nevertheless, a separate MOE analysis was conducted here to characterize the risks associated with AMPA exposure. The NOAEL of 400 mg/kg body wt/day in the subchronic rat study is considered to be the most appropriate value for use in this risk assessment. As noted previously, AMPA was also assessed as a component of the test material used in the glyphosate reproduction and chronic/oncogenicity studies. The lowest NOAEL established in these studies was 2.8 mg/kg body wt/day for chronic effects. This value was also used in the MOE analysis to provide a very conservative estimate of the overall no-effect level for this material.

POEA

The lowest NOAEL of 15 mg/kg body wt/day was selected as a reference point for risk assessment purposes; this value was based on maternal toxicity in the rat developmental toxicity study. As noted above with glyphosate, calculation of an MOE for children based on a NOAEL for maternal toxicity is not biologically relevant. Therefore, the MOE was also calculated using the NOEL of 36 mg/kg body wt/day from the subchronic rat study.

Estimation of Risks to Humans from Acute or Chronic Exposure

The potential risks to humans resulting from exposure to glyphosate, AMPA, and POEA were determined for pesticide applicators and farm children age 1 to 6 years. Applicators were selected because they have the highest potential for exposure among adult subpopulations. The children were selected because they receive the highest dietary intake of all subpopulations on a milligram per kilogram of body weight per day basis and are considered to represent a sensitive subpopulation. Chronic risks were evaluated using a MOE analysis in which MOE values for each of the three substances were calculated by dividing the applicable NOAEL by the estimates of maximum chronic human exposure (Table 9). To assess acute risks, oral LD₅₀ values in rats were divided by estimates of maximum acute human exposure. All MOE values were rounded to three significant figures. Determination of an acceptable MOE relies on the judgment of the regulatory authority and varies with such factors as nature/severity of the toxicological endpoint observed, completeness of the database, and size of the exposed population. For compounds which have a substantial toxicological database, MOE values of 100 or more are generally considered to indicate that the potential for causing adverse health effects is negligible.

Glyphosate

Chronic exposure. In children, the exposure resulting from ingestion of glyphosate residues in food and water was calculated to be 0.052 mg/kg body wt/day. Exposure to professional applicators, which included exposure resulting from the spraying operation along with dietary intake, was estimated to be 0.0323 mg/kg body wt/day. Comparison of these values to the NOAEL of 175 mg/kg body wt/day based on maternal toxicity in the rabbit developmental toxicity study produced MOEs of 3370 and 5420 in children and adults,

TABLE 9
Summary of No-Observed-Adverse-Effect Levels (NOAEL), Worst-Case Exposure Estimates, and Margins of Exposure (MOE) for Glyphosate, AMPA, and POEA

Chemical	NOAEL (mg/kg/day)	Basis of NOAEL	Worst-case chronic exposure (mg/kg/day)		Margin of exposure ^a	
			Adults	Children	Adults	Children
Glyphosate	175	Maternal toxicity in developmental toxicity study			5,420	3,370
	209	90-day rat study	0.0323	0.052	∅	4,020
AMPA	400	90-day rat and developmental toxicity studies			83,300	38,500
	. 2.8	Based on AMPA content in glyphosate used for chronic rat study	0.0048	0.0104	. 583	. 269
POEA	15	Maternal toxicity in developmental toxicity study			461	577
	36	90-day rat study	0.0325	0.026	∅	1380

^a All MOE values rounded to three significant figures.

respectively. Using the more biologically relevant NOAEL of 209 mg/kg body wt/day from the subchronic rat study, the MOE for children was 4020.

Acute exposure. Total acute exposure for children living on a farm was estimated by adding incidental exposure (e.g., reentry, bystander, consumption of sprayed wild foods, swimming in a pond) to that resulting from normal dietary intake as described above. The resulting exposure value was 0.097 mg/kg body wt/day. For applicators, the corresponding aggregate acute exposure value was calculated to be 0.125 mg/kg body wt/day. The acute exposure calculation utilized peak dermal and inhalation measurements (instead of the mean value used for chronic exposure calculations) and included significant exposure from the consumption of sprayed wild foods. The oral LD₅₀ of glyphosate is greater than 5000 mg/kg. The acute exposure values for both children and adult applicators are approximately 40,000 to 50,000 times lower than this value, indicating an extremely low potential for acute toxicity.

AMPA

Chronic exposure. The only significant source of AMPA exposure could occur from ingestion of treated crops in which the plant/bacterial metabolite has been formed. Herbicide application does not result in exposure to AMPA, and the metabolite does not occur to an appreciable degree in water. The chronic exposure estimates for AMPA were calculated to be 0.0104 mg/kg body wt/day for children and 0.0048 mg/kg body wt/day for adults. MOEs were calculated using the definitive

NOAEL of 400 mg/kg body wt/day from the subchronic rat study and the lowest estimated NOAEL (. 2.8 mg/kg body wt/day) derived from long-term studies with glyphosate. The corresponding MOEs are . 269 to 38,500 for children and . 583 to 83,300 for adult applicators.

Acute exposure. Individuals are not exposed to AMPA as bystanders or via reentry into sprayed areas, and levels of the metabolite in water are negligible. Therefore, acute exposure estimates are identical to chronic scenarios and were calculated to be 0.0104 mg/kg body wt/day for children and 0.0048 mg/kg body wt/day for adults. Based on the oral LD₅₀ value of 8300 mg/kg, acute MOEs for children and adults are 798,000 and 1,730,000, respectively.

POEA

Chronic exposure. Aggregate exposure was calculated to be 0.026 mg/kg body wt/day in children and 0.0325 mg/kg body wt/day in adult applicators. The ingestion of food residues accounted for virtually all of the exposure in children, while dermal/inhalation exposure resulting from the spraying operation was the predominant pathway contributing to applicator exposure. Based on the NOAEL of 15 mg/kg body wt/day for maternal toxicity in the rat developmental study, MOEs were determined to be 577 and 461 in children and adults, respectively. When the more biologically relevant NOAEL of 36 mg/kg body wt/day from the subchronic rat study was used, the resulting MOE for children was calculated to be 1380.

Acute exposure. Estimates of aggregated acute exposure in adult applicators (0.163 mg/kg body wt/day) and children (0.0911 mg/kg body wt/day) were substantially higher than those for chronic exposure. In children, this increase was primarily due to contributions from reentry exposure and, to a lesser degree, the ingestion of wild foods. The acute oral LD₅₀ of POEA is approximately 1200 mg/kg. The estimated acute exposure values are 7360 to 13,200 times lower than this value.

OVERALL CONCLUSIONS AND SUMMARY STATEMENT

This assessment was conducted for adult applicators and children (age 1 to 6 years) because they have the highest potential exposures. Estimates of exposure described for these two subpopulations and used in these risk calculations are considered excessive compared to those likely to result in the general population from the use of Roundup herbicide. MOE analyses compare the lowest NOAELs determined from animal studies to worst-case levels of human exposure. MOEs of greater than 100 are considered by authoritative bodies to indicate confidence that no adverse health effects would occur (WHO, 1990). The MOEs for worst-case chronic exposure to glyphosate ranged from 3370 to 5420; the MOEs for AMPA ranged from greater than 269 to 83,300; and for POEA the MOEs ranged 461 to 1380. Based on these values, it is concluded that these substances do not have the potential to produce adverse effects in humans. Acute exposures to glyphosate, AMPA, and POEA were estimated to be 7360±1,730,000 times lower than the corresponding LD₅₀ values, thereby demonstrating that potential acute exposure is not a health concern. Finally, under the intended conditions of herbicide use, Roundup risks to subpopulations other than those considered here would be significantly lower. It is concluded that, under present and expected conditions of new use, there is no potential for Roundup herbicide to pose a health risk to humans.

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Rheumatoid Arthritis among Women in the Agricultural Health Study: Risk Associated with Farming Activities and Exposures

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PURPOSE: Farming has been associated with increased risk of rheumatoid arthritis (RA) in some studies, but specific causes have not been identified. We studied risk factors for RA in the Agricultural Health Study, a cohort of over 57,000 licensed pesticide applicators and their spouses.

METHODS: We used a nested case-control design, limited to female participants. Physician-confirmed cases ($n = 135$) were matched to five controls each ($n = 675$) by birth date. We used logistic regression, adjusting for birth date and state to examine associations, as estimated by odds ratios (OR) and 95% confidence intervals (CI).

RESULTS: Risk of RA was not associated with mixing or applying pesticides overall or with any pesticide class, nor did it vary by number of days or years of use. Certain pesticides were associated with small non-significantly increased risks, including lindane (OR 1.8, 95% CI: 0.6–5.0). RA risk was associated with welding (OR 2.1, 95% CI: 0.8–5.4), albeit imprecisely, but not with solvents or sunlight.

CONCLUSIONS: We did not identify any strong risk factors for RA. Because of the severe disability associated with this relatively common disease, further investigation into causes is warranted both in the Agricultural Health Study and elsewhere.

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INTRODUCTION

Rheumatoid arthritis (RA) affects approximately 1% of the United States population, and as many as 2%–3% of those over age 60 (1, 2). Women are more likely to be affected than men, for unknown reasons (3). The course of the disease varies widely, but is generally associated with progressive disability and early mortality (1).

Several epidemiologic studies have reported increased risk of RA among farmers (4–8). Use of pesticides has been associated with slightly increased risk of RA (20%–30% increases) (4, 6); however, specific pesticides have not been studied epidemiologically. The prevalence of anti-nuclear antibodies (ANAs), a serologic expression of autoimmunity that is not specific to RA, has been associated with residence on a farm among women, as well as with

exposure to insecticides including organochlorines, carbamates, and pyrethroids, and exposure to phenoxyacetic acid herbicides (9). Several organophosphate insecticides have been implicated as having toxicologic properties relevant to systemic autoimmunity, including malathion (10) and chlorpyrifos (11).

Case ascertainment and exposure assessment are major difficulties in studying farming exposures as potential causes of RA. Self-reporting of RA has been shown to be extremely unreliable in other studies, with confirmation as low as 21%–22% (12, 13). With case ascertainment often requiring extensive validation through medical records, case groups for epidemiologic studies tend to be small. Farming occupation and farm residence are not common in most study populations, and exposures to specific pesticides are even less frequent. The combination of small case groups and infrequent exposures can hinder the informativeness of such studies.

The Agricultural Health Study, a cohort of licensed pesticide applicators and their spouses in Iowa and North Carolina, provides an excellent opportunity to study the health effects of pesticide exposures. Because this population affords a large number of persons involved in farming, the effects of pesticides can be investigated in relation to relatively rare diseases. We investigated pesticide use and other farm-related activities and exposures as possible risk

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Selected Abbreviations and Acronyms

ANA Z antinuclear antibody
CI Z confidence interval
DDT Z dichlorodiphenyltrichloroethane
OR Z odds ratio
RA Z rheumatoid arthritis

factors for RA among women in the Agricultural Health Study.

METHODS

The Agricultural Health Study

Individuals of any age applying for certification to apply restricted use pesticides in Iowa or North Carolina from 1993 to 1997 were invited to enroll in the Agricultural Health Study (14). Approximately 52,000 private applicators, typically farmers, were enrolled (92% of those eligible); the private applicators were primarily male (2.6% female). A take-home packet contained an invitation for the spouse to enroll and a questionnaire. The number of spouses who agreed to participate in the study and completed the questionnaire was 32,347 (76% of those eligible).

Confirmation of RA Cases in the Agricultural Health Study

We validated RA diagnosis among women who had self-reported RA during a phase II (5 year follow-up) interview for the AHS before December 2003 (24,514 women had responded to this interview). The phase II interview for women (administered to both applicators and spouses) contained several questions about RA symptoms, testing, and age at diagnosis. We targeted the RA validation effort to subgroups of women based on issues bearing on the feasibility of obtaining physician confirmation (e.g., time since diagnosis ! 10 years vs. longer), and issues reflecting the likelihood that the women truly had RA (e.g., reported

ever having a positive "blood test for RA" [i.e., rheumatoid factor], or reported more than one autoimmune disease). Women who did not report having ever had joint swelling for > 6 weeks (a hallmark symptom of RA and one of the American College of Rheumatology criteria for RA diagnosis) were infrequently selected unless they had another factor indicating probable RA (e.g., positive blood test). The 594 women included in the validation effort, and their inclusion subgroups, are shown in the Appendix. We did not include men in the initial validation because of the lack of information with which to target validation efforts, as men in the Agricultural Health Study were not asked specific questions about RA, such as symptoms and tests.

We recontacted each woman to obtain information about her RA diagnosis and to request signed consent to contact her physicians. For women who self-confirmed their RA diagnosis in the validation interview, we requested information by mail from up to three physicians: (1) the woman's regular physician, who was providing treatment for RA; (2) a rheumatologist (if the regular physician was not a rheumatologist, but there was a rheumatologist that the woman had seen in the past 5 years); and (3) the physician who made the diagnosis of RA (if different from those in 1 and 2). Specific information from the woman's medical history pertaining to RA was elicited from physicians in the form of a checklist, including information on the patient's diagnosis and presence of the American College of Rheumatology classification criteria for RA (15). A woman was classified as having "physician-confirmed" RA if any of her physicians (1) indicated that she had RA by a "yes/no" response, or (2) indicated the presence of at least 4 of 7 American College of Rheumatology classification criteria (15).

Of the 594 women included in the validation process, 136 (23%) cases were physician-confirmed (Table 1). Of the women for whom we received information from any of their physicians (n Z 186), RA diagnosis was confirmed for 73%. Thirty-three of the physician-confirmed cases (24.3%) were incident between the baseline and phase II

TABLE 1. Validation of female RA cases in the Agricultural Health Study cohort

	Iowa	North Carolina	Total
Final status of total in validation effort*	n (% of 358) ^y	n (% of 236) ^y	n (% of 594) ^y
Did not complete validation interview	32 (9.0)	30 (12.7)	62 (10.4)
Diagnosis not confirmed in validation interview	120 (33.5)	73 (30.9)	193 (32.5)
Diagnosis confirmed in validation interview but consent for physician contact not received	62 (17.3)	67 (28.4)	129 (21.7)
Diagnosis confirmed in validation interview but no response from any physician	13 (3.6)	11 (4.7)	24 (4.0)
Diagnosis confirmed in validation interview but physician did not confirm diagnosis	38 (10.6)	12 (5.1)	50 (8.4)
Physician-confirmed RA ^z	93 (26.0)	43 (18.2)	136 (22.9)

*Each group is mutually exclusive.

^yTotal includes those women who self-reported RA in the baseline or phase II interviews who were contacted for validation; does not include all self-reported RA in Agricultural Health Study.

^zPhysician-confirmed by 'yes/no' question about diagnosis or by report of 4 of 7 American College of Rheumatology classification criteria.

interview. For prevalent cases, the duration between the diagnosis and the baseline interview was less than 5 years for 30 cases (22.1%), from 5 to within 10 years for 25 cases (18.4%), and 10 years or longer for 48 cases (35.3%). The ages at the time of diagnosis for physician-confirmed cases ranged from 1 to 73 years (Table 2). Sixty-four percent of the physician-confirmed cases had tested positive for rheumatoid factor (as reported by either the woman or her physician), which is similar to other populations (16, 17).

We considered a more liberal definition of RA than physician-confirmed only, such as inclusion of all 339 women who self-confirmed their RA diagnosis during the validation interview. Of those women, 210 (61.9%) provided consent for us to contact their physicians. Upon further examination of heterogeneity within the self-reported RA cases, we suspected that women who did not provide consent for physician contact included false positive cases, as they were significantly less likely to have been diagnosed by or ever treated by a rheumatologist, were less likely to be currently taking prescription medications for RA, and reported having ever taken fewer different prescription medications for RA than those who consented to physician contact. Based on these discrepancies, we decided to limit our definition of confirmed RA to the "gold standard" of physician-confirmed cases.

Nested Case-Control Study Population

Physician-confirmed RA cases formed the case group for our case-control analysis. Because most of the exposures of interest were relevant to adulthood, we excluded one confirmed case who had been diagnosed at 1 year of age. Cases ($n = 135$) were matched to five controls each ($n = 675$) by birth date, within one year. Controls were selected from among women in the Agricultural Health Study who had completed the phase II interview ($n = 24,514$; the same group from which cases were validated), except those who had reported any systemic autoimmune disease (RA, scleroderma,

systemic lupus erythematosus, or Sjögren's syndrome) at either the baseline or phase II interview. The rationale behind this exclusion was based on the fact that we did not attempt to validate every participant who self-reported RA diagnosis, in addition to the difficulty of diagnosis and potential etiologic overlap of systemic autoimmune diseases.

Exposure Information

Information on demographics, farming history, pesticide use, and residential, lifestyle, and occupational factors were available from the Agricultural Health Study questionnaires administered at the baseline (questionnaires are available at www.aghealth.org, last accessed September 19, 2005). The questionnaire elicited information about pesticides that were ever personally mixed or applied by the respondent in their lifetime for use on the farm, in the home or garden, or in commercial application (without designating between these different uses). Information of interest included use of 49 specific pesticides (note: permethrin formulations for crop and animal applications were categorized together), and duration and frequency of use for all pesticides combined. Other factors of interest included farming activities and exposures in the longest job held off the farm.

Statistical Analyses

Associations between exposures and the risk of RA were estimated using unconditional logistic regression to generate odds ratios (OR) and 95% confidence intervals (CI), adjusting for birth date (the matching factor) and state (Iowa or North Carolina). After consideration of pack-years of cigarette smoking (0, 0.25-19.5, and > 20 pack-years) as a potential confounder in all models, estimates were not importantly affected ($< 10\%$ change); thus, final models do not contain the smoking variables.

Several subanalyses were conducted. To evaluate the internal consistency of our results, we conducted analyses stratified by state. Analyses limited to incident cases who were diagnosed within or after the year of enrollment in the Agricultural Health Study ($n = 33$), and all controls were conducted to check that any observed associations persisted in models in which the exposure was reported prior to disease diagnosis. Similarly, analyses limited to cases who were diagnosed within 5 years before or anytime after their year of enrollment in the Agricultural Health Study ($n = 63$), and all controls were conducted to check that any observed associations persisted in models for which the reported "ever" exposure was more likely to have occurred prior to disease diagnosis than for more distantly diagnosed cases. Analyses stratified by rheumatoid factor positivity were conducted to evaluate whether any observed associations were limited to one case group.

TABLE 2. Characteristics of female RA cases* in the Agricultural Health Study

Characteristic	Cases ($n = 136$)
	n (%)
Age at diagnosis (years)	
≤ 25	8 (5.9)
25-39	39 (28.7)
40-54	53 (39.0)
55-69	33 (24.3)
70 or older	3 (2.2)
Rheumatoid factor	
Positive	87 (64.0)
Negative	49 (36.0)

*Physician-confirmed cases.

RESULTS

Of the physician-confirmed RA cases included in the nested case-control study, the median age at diagnosis was 47 years. Only 4 cases and 24 controls were applicators; the remainder were spouses. There were few demographic differences between cases and controls (Table 3), although cases were slightly more likely to reside in North Carolina (OR Z 1.3). The risk of RA was associated with pack-years of smoking, with 60% increased risk among women who smoked 20 pack-years or more; however, the risk did not follow a monotonic trend. The pack-years effect was confined to past smokers (for the comparison of 20 or more pack years with never smoked, OR Z 2.8, 95% CI: 1.1-7.0 among past smokers and OR Z 1.0, 95% CI: 0.4-2.6 among current smokers). Current smokers were at slightly increased risk of RA (OR Z 1.3) when pack-years were not taken into account, whereas past smokers were not. RA cases were more likely than were controls to be currently overweight (OR Z

1.4, 95% CI: 0.9-2.1), which could be a consequence of disability resulting from the disease. Living on a farm before age 18 was not associated with the risk of RA, nor did the risk vary by duration or age at the time of farm residence.

RA risk was not associated with having ever personally mixed or applied pesticides (Table 4), nor did it vary by the number of days or years a woman participated in these activities. Use of herbicides was not associated with RA risk, nor were insecticides overall or the broad insecticide groupings of organophosphates, organochlorines, or carbamates. Elevated ORs were observed for some of the 49 specific pesticides (full set of results not shown), although no estimate was statistically significant, including the insecticides lindane (OR Z 1.8), toxaphene (OR Z 2.3), malathion (OR Z 1.3), DDVP (OR Z 1.4), and the herbicide imazethapyr (OR Z 1.5). DDT use was not associated with the risk of RA (OR Z 1.0). The broad grouping of phenoxyacetic acid herbicides was associated with a significantly decreased risk of RA (OR Z 0.5), specifically due to an association with 2,4-D (other herbicides included in this category were 2,4,5-T and MCPA); several other major herbicide classes and specific herbicides showed similar inverse associations with RA, including triazines and thiocarbamates.

Most nonpesticide farming activities and other occupational exposures were not associated with RA (Table 5). 85% of women in the study had worked in jobs off the farm. Elevated odds ratios were observed for both welding on the farm and in off-farm jobs, based on small numbers of exposed subjects, and any welding was associated with 2.1-fold increased risk of RA (6 exposed cases, 16 controls, 95% CI: 0.8-5.4). Elevated, but imprecise odds ratios were also observed for exposure in off-farm jobs to engine exhaust, gasoline, solder, drills, and silica dust. There was virtually no association with sunlight exposure, reported as the number of hours per day spent in the sun during the growing season 10 years prior to the baseline interview.

Some associations differed by state of residence (Table 6). Smoking was associated with increased risk in both states for 20 or more pack-years of cigarettes, although the association was stronger in North Carolina than Iowa. There was some indication that insecticides as a group were associated with increased risk of RA in North Carolina (OR Z 1.9) but not Iowa (OR Z 1.0). Broad groupings of insecticides associated with elevated ORs in North Carolina included organochlorines and carbamates, although the increases were not statistically significant. Specific pesticides with elevated odds ratios only among North Carolina residents included DDT (OR Z 1.8, 95% CI: 0.6-5.2), carbaryl (OR Z 2.2, 95% CI: 1.1-4.5), and malathion (OR Z 1.6, 95% CI: 0.8-3.5). The modest increased risks observed among the entire study population for welding and lindane were consistent in both states, while data were too sparse to compare

TABLE 3. Demographic and lifestyle factors and the risk of RA among women in the Agricultural Health Study

Characteristic	Cases (n Z 135)*	Controls (n Z 675)	OR (95% CI) ^z
	n (%) ^y	n (%) ^y	
State of residence			
Iowa	92 (68.2)	494 (73.2)	1.0
North Carolina	43 (31.9)	181 (26.8)	1.3 (0.9-1.9)
Race			
White	131 (97.8)	649 (97.9)	1.0
Non-White	3 (2.2)	14 (2.1)	0.9 (0.3-3.3)
Education			
Did not finish high school	10 (7.5)	21 (3.2)	1.0
High school graduate	52 (38.8)	278 (42.1)	0.8 (0.5-1.3)
Some college	36 (26.9)	178 (26.9)	0.9 (0.5-1.5)
College graduate or more	22 (16.4)	109 (16.5)	0.8 (0.4-1.6)
Cigarette smoking			
Never	94 (72.9)	493 (76.2)	1.0
Past smoker	23 (17.8)	109 (16.9)	1.1 (0.6-1.8)
Current smoker	12 (9.3)	45 (7.0)	1.3 (0.7-2.6)
Pack-years of cigarettes			
0	94 (73.4)	494 (77.2)	1.0
0.25 to 19.5	21 (16.4)	107 (16.7)	1.0 (0.6-1.7)
> 20	13 (10.2)	39 (6.1)	1.6 (0.8-3.2)
Overweight (BMI >25) ^x	66 (61.7)	257 (54.0)	1.4 (0.9-2.1)
Years lived or worked on a farm			
0 to 10	7 (5.4)	36 (5.6)	1.0
11 to 30	41 (31.5)	190 (29.4)	1.2 (0.5-3.0)
> 30	82 (63.1)	420 (65.0)	1.1 (0.4-2.6)
Lived on farm for			
More than 10 years	87 (66.4)	410 (63.8)	1.1 (0.8-1.7)
Before age 18			

*The nested case-control study included all physician-confirmed cases except one who was diagnosed in infancy.

^yFrequencies of characteristics do not always sum to the total number of cases and controls because of missing data.

^zAll estimates are adjusted for birth date (matching factor) and state.

^xBody mass index (BMI) Z (weight in kilograms)/(height in meters)².

TABLE 4. Agricultural pesticide exposures and the risk of RA among women in the Agricultural Health Study

Characteristic	Cases (n Z 135)*	Controls (n Z 675)	OR (95% CI) [‡]
	n (%) [‡]	n (%) [‡]	
Personally mixed or applied pesticides (ever)	88 (65.7)	417 (62.9)	1.2 (0.8-1.7)
Years personally mixed or applied pesticides			
0	46 (40.0)	246 (44.7)	1.0
1 to 10	27 (23.5)	118 (21.4)	1.2 (0.7-2.0)
O 10	34 (29.6)	161 (29.2)	1.1 (0.7-1.7)
Days per year personally mixed or applied pesticides			
0	46 (40.0)	246 (44.4)	1.0
1 to 9	53 (46.1)	222 (40.1)	1.3 (0.8-2.0)
O 9	16 (13.9)	86 (15.5)	1.0 (0.5-1.8)
Type of pesticides used			
Herbicides	54 (40.6)	253 (38.9)	1.1 (0.8-1.6)
Insecticides	66 (50.0)	301 (45.8)	1.2 (0.8-1.7)
Fungicides	4 (3.1)	33 (5.1)	0.5 (0.2-1.6)
Carbamates	52 (39.4)	231 (35.2)	1.2 (0.8-1.7)
Organochlorines	14 (10.9)	63 (10.0)	1.1 (0.6-2.0)
Organophosphates	47 (35.6)	205 (31.3)	1.2 (0.8-1.8)
Phenoxyacetic acids	12 (9.2)	115 (18.0)	0.5 (0.3-0.9)
Thiocarbamates	3 (2.3)	33 (5.1)	0.4 (0.1-1.4)
Triazines	4 (3.1)	44 (6.9)	0.5 (0.2-1.3)
Specific pesticides [‡]			
2,4-D	12 (9.2)	112 (17.6)	0.5 (0.3-0.9)
Alachlor	3 (2.3)	31 (5.0)	0.5 (0.1-1.6)
Atrazine	4 (3.1)	37 (5.8)	0.5 (0.2-1.6)
Carbaryl	50 (38.8)	219 (34.3)	1.2 (0.8-1.8)
Chlordane	3 (2.4)	31 (5.0)	0.5 (0.1-1.6)
Chlorpyrifos	6 (4.7)	35 (5.6)	0.8 (0.4-2.1)
Coumaphos	2 (1.6)	13 (2.1)	0.8 (0.2-3.5)
Cyanazine	2 (1.6)	14 (2.2)	0.8 (0.2-3.4)
DDT	8 (6.5)	38 (6.2)	1.0 (0.4-2.2)
DDVP	5 (3.9)	19 (3.0)	1.4 (0.5-3.9)
Diazinon	12 (9.5)	64 (10.3)	0.9 (0.5-1.7)
Glyphosate	52 (39.1)	222 (34.5)	1.2 (0.8-1.8)
Imazethapyr	5 (3.9)	18 (2.9)	1.5 (0.5-4.1)
Lindane	5 (4.0)	14 (2.3)	1.8 (0.6-5.0)
Malathion	36 (28.4)	150 (23.6)	1.3 (0.8-2.0)
Maneb	3 (2.4)	16 (2.5)	0.8 (0.2-3.0)
Metolachlor	2 (1.6)	26 (4.2)	0.4 (0.1-1.7)
Permethrin	7 (5.3)	35 (5.4)	1.0 (0.4-2.3)
Phorate	2 (1.6)	11 (1.8)	0.9 (0.2-4.3)
Terbufos	3 (2.4)	16 (2.6)	1.0 (0.3-3.4)
Toxaphene	2 (1.6)	4 (0.7)	2.3 (0.4-12.9)

*The nested case-control study included all physician-confirmed cases except one that was diagnosed in infancy.

[‡]Frequencies of characteristics do not always sum to the total number of cases and controls because of missing data.[‡]All estimates are adjusted for birth date (matching factor) and state.[‡]Specific pesticides were presented where the case exposure frequency was O 1%; the total number of specific pesticides examined was 49.

risks for DDVP, toxaphene, or imazethapyr. There was some indication of an inverse association with 2,4-D in both states, although the exposure frequency in North Carolina was much lower than in Iowa.

TABLE 5. Nonpesticide occupational activities and exposures and the risk of RA among women in the Agricultural Health Study

Characteristic	Cases (n Z 135)*	Controls (n Z 675)	OR (95% CI) [‡]
	n (%) [‡]	n (%) [‡]	
Farming activities [‡]			
Milk cows	3 (2.4)	17 (2.7)	0.9 (0.3-3.2)
Drive trucks	46 (35.9)	215 (33.4)	1.1 (0.8-1.7)
Drive diesel tractors	32 (25.2)	235 (36.4)	0.6 (0.4-1.0)
Drive gasoline tractors	33 (25.8)	178 (27.6)	1.0 (0.6-1.5)
Weld	2 (1.6)	5 (0.8)	2.3 (0.4-11.8)
Grind animal feed	6 (4.7)	31 (4.8)	1.1 (0.4-2.6)
Use gasoline for cleaning	17 (13.3)	114 (17.8)	0.8 (0.4-1.3)
Use other solvents for cleaning	27 (21.1)	150 (23.4)	0.9 (0.6-1.5)
Paint	40 (30.5)	223 (34.7)	0.9 (0.6-1.4)
Contact with animal blood	13 (10.1)	78 (12.1)	0.9 (0.5-1.7)
Hours per day spent in sun during growing season (10 years ago)			
! 1	15 (14.3)	77 (16.2)	1.0
1-2	25 (23.8)	120 (25.3)	1.1 (0.5-2.2)
3-5	39 (37.1)	176 (37.1)	1.1 (0.6-2.2)
6 C	26 (24.8)	102 (21.5)	1.2 (0.6-2.5)
Occupational exposures in job held off the farm [‡]			
Ever had a job off the farm	113 (86.3)	550 (85.0)	1.1 (0.6-1.9)
Asbestos	6 (4.6)	37 (5.7)	0.8 (0.3-2.0)
Cotton dust	2 (1.5)	17 (2.6)	0.5 (0.1-2.1)
Engine exhaust	9 (6.9)	34 (5.3)	1.4 (0.7-3.1)
Gasoline	4 (3.1)	15 (2.3)	1.4 (0.5-4.4)
Grain dust	2 (1.5)	15 (2.3)	0.7 (0.2-3.1)
Lead solder	2 (1.5)	7 (1.1)	1.5 (0.3-7.4)
Mineral or mining dust	2 (1.5)	10 (1.6)	1.0 (0.2-4.8)
Pneumatic drill (vibrations)	2 (1.5)	5 (0.8)	2.1 (0.4-11.1)
Silica/sand dust	3 (2.3)	8 (1.2)	1.9 (0.5-7.4)
Solvents	7 (5.3)	54 (8.4)	0.6 (0.3-1.5)
Welding fumes	4 (3.1)	12 (1.9)	1.8 (0.6-5.6)
Wood dust	2 (1.5)	16 (2.5)	0.6 (0.1-2.7)
X-ray radiation	5 (3.8)	37 (5.7)	0.7 (0.3-1.7)

*The nested case-control study included all physician-confirmed cases except one that was diagnosed in infancy.

[‡]Frequencies of characteristics do not always sum to the total number of cases and controls because of missing data.[‡]All estimates are adjusted for birth date (matching factor) and state.[‡]Conducted at least once per month in summer or winter.[‡]Self-reported exposure in longest job held off the farm.

In subanalyses restricted to incident cases (n Z 33) and all controls (results not shown in tables), elevated ORs were estimated for being overweight (OR Z 1.3), 20 or more pack-years of smoking (OR Z 1.6), current smoking (OR Z 1.8), and welding (OR Z 3.1), although all estimates were imprecise due to the very small case group. Elevated odds ratios observed for the entire case group persisted in analyses of incident cases for exposure to the pesticides lindane, toxaphene, DDVP, and imazethapyr. The estimate for malathion was not elevated (OR Z 0.7). An inverse association of the risk of RA with 2,4-D use was again observed in the analyses of incident cases (OR Z 0.2). Very similar

results were observed in the analysis restricted to cases diagnosed within 5 years prior to the baseline interview.

There were no apparent systematic differences in the magnitude of associations with farming exposures between rheumatoid factor positive or negative cases (results not shown). Cases were more likely than controls to be overweight only if they were rheumatoid factor positive (OR 1.7, 95% CI: 1.0-3.0), perhaps as a consequence of the more severe disease course typical of cases testing positive for rheumatoid factor (18).

DISCUSSION

Several reports have observed increased RA incidence among farmers (4-8), and ours is the first to investigate specific pesticides for their associations with RA. We did not find any strong associations between pesticide exposure and the risk of RA, either for pesticides overall or for broad groupings of pesticide type. These results agree with those of Olsson and colleagues (7), in which RA risk was associated with farming occupation, but not with self-reported pesticide use, and with two other previous studies that found only slightly increased risks (effect estimates of 1.2 and 1.3) associated with pesticide use (4, 6). There was some suggestion in our study that a few specific pesticides may contribute to increased risk of RA; however, we did not have sufficient power to detect these modest increased risks. We had adequate statistical power to detect moderate risks of approximately 2-fold increase (or, conversely, 50% decreased risk) for exposures with at least 10% exposure frequency among controls, as evidenced by our observation of an inverse association with 2,4-D herbicide. Thus, our study was capable of detecting risks of this magnitude for the broad groupings of insecticides and herbicides; the pesticide classes organochlorines; organophosphates; carbamates; and the specific pesticides malathion, carbaryl, diazinon, and glyphosate. The fact that these estimates were not significantly elevated speaks to the probable magnitude of the true associations.

Several major-use herbicides were inversely associated with RA and significantly so for 2,4-D, and similarly for atrazine and alachlor. There have been no previous reports about the potential of these herbicides to contribute to autoimmunity. Suspected immune effects of 2,4-D include immunosuppression (19, 20) but studies have not focused on the broad spectrum of immune effects including autoimmunity. The similar magnitude of the risk deficits for these herbicides suggests a possible bias resulting from the likely reduction of the women's involvement in farm activities such as pesticide application following disease onset; however, the weakly elevated risk estimates observed for other

major-use herbicides such as glyphosate and imazethapyr contradict this theory.

Organochlorine insecticide use was not associated with RA; however, we observed imprecise elevated odds ratios for specific organochlorines, including lindane and toxaphene. We did not observe an association with DDT overall, although there was a moderate, but not-statistically significant, increased risk in North Carolina. It is possible that another risk factor for RA is linked with use of some insecticides in North Carolina (such as silica content of soil in some regions in which specific crops are grown), which could explain why no increased risk was observed in Iowa. North Carolina subjects were on average older than those from Iowa (35% vs. 22% of subjects > 60 years of age at baseline), and an alternate possibility is that their exposures occurred during an important time window for RA etiology. Chance is also a likely explanation for observed differences between the states.

Evaluation of specific organophosphates indicated weak (not statistically significant) associations with malathion and DDVP. Several experimental studies in animals have indicated effects of malathion on the immune system (21). A study of the insecticide malathion in lupus-prone mice found that oral administration accelerated disease onset and increased the levels of factors indicating autoimmunity including protein in urine, rheumatoid factor, and anti-dsDNA antibody in serum (10); these biologic responses are also relevant to RA. Nevertheless, the inconsistency of the malathion-RA association between states and the non-elevated OR for the association between malathion and incident RA in our study detract from the credibility of the association. Chlorpyrifos was not associated with RA, providing no support for a previous observation of frequent autoantibodies observed among a group of chlorpyrifos-exposed people (11); however, human exposure to chlorpyrifos occurs from multiple sources including diet, and it is possible that our exposure measure did not capture the true variability in exposure.

Farming exposures other than pesticides may be relevant to autoimmune etiology. Other farming exposures including sunlight, dusts (e.g., grain, silica), nonpesticide chemical exposures (e.g., solvents), and viruses have not been extensively researched in relation to autoimmune diseases. We observed an increased risk of RA associated with welding, whether it occurred on or off the farm. This association, although not statistically significant, was observed in both states and in all subanalyses and is in agreement with a previous study that found an 80% increased risk of RA associated with occupation as a mechanic, repairer, sheet metal worker, or welder (7). Several metals that are typical components of welding fumes have been reported to have immune-related effects. Cadmium has been observed to cause antinuclear antibodies in mice upon exposure (22),

TABLE 6. State-specific associations between selected pesticide and other exposures and the risk of RA among women in the Agricultural Health Study (odds ratios [OR] and 95% confidence intervals [CI])

Characteristic	Iowa			North Carolina		
	Cases (n Z 92)	Controls (n Z 494)	OR (95% CI) ^y	Cases (n Z 43)	Controls (n Z 181)	OR (95% CI) ^y
	n (%) [*]	n (%) [*]		n (%) ^y	n (%) [*]	
Pack-years of cigarettes						
0	69 (79.3)	379 (79.5)	1.0	25 (61.0)	115 (70.6)	1.0
0.25 to 19.5	13 (14.9)	77 (16.1)	0.9 (0.5-1.7)	8 (19.5)	30 (18.4)	1.2 (0.5-3.0)
> 20	5 (5.8)	21 (4.4)	1.3 (0.5-3.7)	8 (19.5)	18 (11.0)	2.0 (0.8-5.2)
Years lived or worked on a farm						
0-10	5 (5.6)	18 (3.7)	1.0	2 (4.9)	18 (11.4)	1.0
11-30	30 (33.7)	154 (31.6)	0.7 (0.2-2.1)	11 (26.8)	36 (22.8)	2.6 (0.5-13.1)
> 30	54 (60.7)	316 (64.8)	0.7 (0.2-2.0)	28 (68.3)	104 (65.8)	2.1 (0.4-9.8)
Personally mixed or applied pesticides (ever)	60 (65.9)	326 (66.3)	1.0 (0.6-1.6)	28 (65.1)	91 (53.2)	1.8 (0.9-3.5)
Years personally mixed or applied pesticides						
0	31 (39.7)	166 (41.3)	1.0	15 (40.5)	80 (52.6)	1.0
1-10	40 (51.3)	175 (43.5)	1.2 (0.7-2.2)	5 (13.5)	27 (17.8)	1.1 (0.3-3.2)
> 10	7 (9.0)	61 (15.2)	0.9 (0.5-1.6)	14 (37.8)	38 (25.0)	1.8 (0.8-4.0)
Days per year personally mixed or applied pesticides						
0	31 (39.7)	166 (41.6)	1.0	15 (40.5)	80 (52.6)	1.0
1 to 9	22 (28.2)	91 (22.8)	1.2 (0.7-2.1)	13 (35.1)	47 (30.9)	1.6 (0.7-3.8)
> 9	20 (25.6)	123 (30.8)	0.6 (0.3-1.5)	9 (24.3)	25 (16.5)	2.0 (0.8-5.3)
Type of pesticides used						
Insecticides	42 (46.2)	226 (46.4)	1.0 (0.6-1.6)	24 (58.5)	75 (44.1)	1.9 (0.9-3.8)
Herbicides	40 (44.0)	203 (41.9)	1.1 (0.7-1.7)	14 (33.3)	50 (30.1)	1.3 (0.6-2.7)
Fungicides	1 (1.1)	14 (2.9)	0.4 (0.1-2.8)	3 (7.7)	19 (11.4)	0.7 (0.2-2.4)
Organochlorines	6 (6.8)	45 (9.6)	0.7 (0.3-1.8)	8 (20.0)	18 (11.2)	1.9 (0.8-4.8)
Organophosphates	32 (35.2)	151 (31.1)	1.2 (0.8-2.0)	15 (36.6)	54 (32.0)	1.2 (0.6-2.5)
Carbamates	30 (33.0)	165 (33.7)	1.0 (0.6-1.6)	22 (53.7)	66 (39.5)	1.9 (0.9-3.8)
Thiocarbamates	1 (1.1)	20 (4.1)	0.3 (0.04-2.0)	2 (4.8)	13 (7.9)	0.6 (0.1-2.7)
Phenoxyacetic acids	12 (13.3)	102 (21.5)	0.6 (0.3-1.1)	0 (0.0)	13 (7.9)	^z
Triazines	4 (4.4)	40 (8.5)	0.5 (0.2-1.5)	0 (0.0)	4 (2.4)	^z
Specific pesticides						
2,4-D	12 (13.3)	99 (20.9)	0.6 (0.3-1.1)	0 (0.0)	13 (7.9)	^z
Carbaryl	28 (31.8)	160 (33.5)	0.9 (0.6-1.5)	22 (53.7)	59 (36.7)	2.2 (1.1-4.5)
DDT	2 (2.4)	25 (5.4)	0.4 (0.1-1.9)	6 (15.4)	13 (8.5)	1.8 (0.6-5.2)
DDVP	5 (5.6)	18 (3.8)	1.5 (0.6-4.3)	0 (0.0)	1 (0.6)	^z
Glyphosate	38 (41.8)	174 (36.3)	1.3 (0.8-2.0)	14 (33.3)	48 (29.1)	1.3 (0.6-2.8)
Imazethapyr	5 (5.7)	17 (3.7)	1.6 (0.6-4.4)	0 (0.0)	1 (0.7)	^z
Lindane	3 (3.5)	10 (2.2)	1.7 (0.5-6.3)	2 (5.0)	4 (2.6)	2.0 (0.4-11.6)
Malathion	23 (26.4)	114 (23.9)	1.2 (0.7-2.0)	13 (32.5)	36 (22.6)	1.6 (0.8-3.5)
Toxaphene	0 (0.0)	3 (0.7)	^z	2 (5.1)	1 (0.7)	7.2 (0.6-82.2)
Welding (on or off the farm)	5 (5.6)	15 (3.1)	1.9 (0.7-5.2)	1 (2.3)	1 (0.6)	4.0 (0.2-65.8)

*Frequencies of characteristics do not always sum to the total number of cases and controls because of missing data.

^yAll estimates are adjusted for birth date (matching factor).^zEffect could not be estimated due to sparse data.

and mercury has been shown to have dual immunosuppressive and immunostimulatory effects depending on the dose, which is a common property among agents suspected to cause autoimmune diseases (21).

A previous report suggested a causal association between substantial solvent use and RA (4), but in our study neither farm nor nonfarm solvent exposures were associated with RA. There is a potential for high exposure to crystalline silica in some agricultural activities (23), and silica exposure may be associated with several autoimmune diseases, including RA (24). While we did not have any assessment of silica

on the farm, there was a moderate, albeit imprecise, association with nonfarm silica/sand dust exposure (OR Z 1.9). These results for nonpesticide farming exposures are very preliminary, since the detail of information for these exposures was less than for use of pesticides.

Frequency and duration of cigarette smoking contributed to increased risk among past smokers in our study. These results are consistent with previous studies which also found an increased risk of RA associated with smoking (25-33). When pack-years were not taken into account, the risk of RA was higher for current smoking than for past smoking

in our study and several others (25, 28, 30), an effect that has been hypothesized to result from smoking causing a short-term decrease in estrogen levels. RA symptoms have been observed to be less frequent in patients during pregnancy when endogenous estrogen levels are high (34), and exogenous estrogen has been shown to suppress collagen-induced arthritis (an animal model for RA) in mice (35, 36). However, there are several other possible pathways by which cigarette smoking might contribute to RA etiology, including oxidative stress (37).

The weak-to-modest associations we observed were with crude exposure metrics such as ever use of a pesticide. More detailed information on frequency and duration of use and intensity of exposure to specific pesticides would be necessary to determine whether stronger associations supporting biologic plausibility are present among the highly exposed. The majority of women in our study were spouses of farmers, as opposed to being farmers themselves. Although most spouses in our study reported using pesticides, it is possible that their exposures are less frequent or at a lower volume than are farmers' exposures; it is also possible that their applications were for home or garden use as opposed to applications on crops or animals. Our results may therefore not be generalizable to farmers and other licensed pesticide applicators. Unfortunately, there were too few female licensed applicators included in our study to examine risk patterns among this group separately; however, exclusion of this group did not change our results. Improved exposure data would also include information on the timing of pesticide use and other exposures relative to RA diagnosis, providing some certainty that exposure occurred before diagnosis. Certainty of the timing of exposure relative to diagnosis is particularly important when examining work-related tasks and exposures because of the possible change in activities resulting from disease-related disability (1).

Although the Agricultural Health Study is a large cohort that includes over 30,000 women, we were able to confirm only a relatively small number of RA cases. This limited the power of our study to identify specific risk factors for RA. Our physician-confirmed case group is likely to include cases that were diagnosed more recently than 10 years ago, because we specifically targeted this subgroup for case validation as cases whose validation would be more feasible. Although not representative of all RA cases, recently diagnosed cases may be more relevant for our analyses, in which we were interested in exposures that occurred prior to diagnosis but for which we did not have information on the timing of exposure. There was some indication that our case group was inclusive of more severe RA cases than would be represented in a general population sample. Among self-confirmed RA cases that consented to physician contact, we were more successful in obtaining physician responses for women with self-reported rheumatoid factor positivity than for those without.

It is possible that physicians have greater familiarity with more severe RA cases due to the frequency of patient visits, and were thus more readily able to complete the validation forms. Our results may therefore be more generalizable to a profile of more severe RA than would be encountered in the general population. Nevertheless, our percentage of rheumatoid factor positivity among cases was similar to other study populations of physician-confirmed cases (16).

Despite limitations, our study is the most comprehensive to date in terms of evaluating farming activities and exposures in relation to RA. There is little evidence from this study that pesticides overall or broad pesticide groupings are strongly associated with RA, although our study cannot rule out associations with some specific pesticides. Given the magnitudes of associations with pesticide use observed by us and other researchers, focus should turn toward nonpesticide farming exposures as potential candidates to explain the increased RA incidence observed among farmers. In particular, welding and other metal exposures and silica and other dusts should be studied.

We would like to thank the following people for assisting with validation of RA cases in Iowa and North Carolina: Dr. Charles Lynch, Ms. Patricia Gillette, Mr. Charles Knott, Ms. Joy Pierce, and Dr. Berit Stroehla.

APPENDIX

Subgroups included in rheumatoid arthritis validation effort*

Group ^b	Years since diagnosis	Responses in AHS phase II interview	n in cohort	n in validation effort
1	Any	Reported O 1 autoimmune disease (e.g., RA and either systemic lupus erythematosus, scleroderma, or Sjögren's syndrome)	53	53
2	< 10	Swelling ^c and RA blood test ^x with positive result	97	97
3	< 10	Swelling and RA blood test with negative result	41	41
4	< 10	No swelling and RA blood test with positive result	89	89
5	< 10	Swelling but no RA blood test done	71	57
6	< 10	No swelling and no RA blood test done OR No swelling and RA blood test with negative result	170	50
7	O 10	Swelling and RA blood test with positive result	107	107
8	O 10	EITHER swelling or RA blood test with positive result, not both	103	50
9	Any	Reported RA in phase I but not phase II	558	50

* Among women who responded to the AHS phase II interview (five year follow-up).

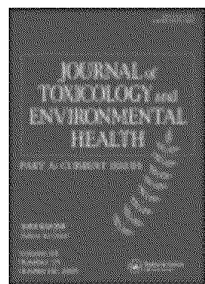
^b All groups are mutually exclusive.

^c Women responded to the question, "Have you ever had swelling in your wrist, finger, elbow, or knee joints that lasted for six weeks or more?"

^x "RA blood test" refers to test for rheumatoid factor.

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Regional Differences in Time to Pregnancy Among Fertile Women from Five Colombian Regions with Different use of Glyphosate

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Regional Differences in Time to Pregnancy Among Fertile Women from Five Colombian Regions with Different use of Glyphosate

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The objective of this study was to test whether there was an association between the use of glyphosate when applied by aerial spray for the eradication of illicit crops (cocaine and poppy) and time to pregnancy (TTP) among fertile women. A retrospective cohort study (with an ecological exposure index) of first pregnancies was undertaken in 2592 fertile Colombian women from 5 regions with different uses of glyphosate. Women were interviewed regarding potential reproductive, lifestyle, and work history predictors of TTP, which was measured in months. Fecundability odds ratios (fOR) were estimated using a discrete time analogue of Cox's proportional hazard model. There were differences in TTP between regions. In the final multivariate model, the main predictor was the region adjusted by irregular relationship with partner, maternal age at first pregnancy, and, marginally, coffee consumption and self-perception of water pollution. Boyacá, a region with traditional crops and, recently, illicit crops without glyphosate eradication spraying (manual

eradication), displayed minimal risk and was the reference region. Other regions, including Sierra Nevada (control area, organic agriculture), Putumayo and Nariño (illicit crops and intensive eradication spray program), and Valle del Cauca, demonstrated greater risk of longer TTP, with the highest risk for Valle del Cauca (fOR 0.15, 95% CI 0.12, 0.18), a sugar-cane region with a history of use of glyphosate and others chemicals for more than 30 yr. The reduced fecundability in some regions was not associated with the use of glyphosate for eradication spraying. The observed ecological differences remain unexplained and may be produced by varying exposures to environmental factors, history of contraceptive programs in the region, or psychological distress. Future studies examining these or other possible causes are needed.

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Glyphosate is one of the most widely used herbicides globally and has been registered for use in Colombia since 1972 for weed control in a wide range of crops and in the process of sugar cane maturation. Beginning in the early 1980s, it was used for eradicating the illegal crops of coca (*Erythroxylum coca*) and poppy (*Papaver somniferum*). Since 2000, it has been more widely used for the eradication of illicit crops. The area of coca sprayed with glyphosate has shown a steady increase over recent years, reaching 153,000 ha in 2007 (personal communication, National Police of Colombia, Bogotá, December 2007). According to Colombian use data, 10–13% of the total amount of glyphosate purchased in the country is used for aerial spraying of illicit crops; the remainder is used in both legal and illegal crop production (Solomon et al., 2007).

Colombia is organized into 32 administrative departments (*departamentos*). In 12 of them, illicit crops have been sprayed with glyphosate by aerial application since 2000. The location and amounts of glyphosate applied for this purpose are accurately

known. Glyphosate is used for other purposes in all departments, but actual use statistics are not known as sales data are not collected.

In developed countries, investigators have increasingly used time to pregnancy (TTP) as a sensitive clinical marker of multiple early adverse reproductive effects (Baird et al., 1986; Joffe 1997, 2000; Joffe & Barnes 2000; Tingen et al., 2004; Joffe et al., 2005). Epidemiological studies examined the role of agriculture and pesticide exposure in reducing the probability of achieving conception in a menstrual cycle (also known as fecundability) with mixed results (De Cock et al., 1994; Larsen et al., 1998; Curtis et al., 1999; Thonneau et al., 1999; Abell et al., 2000; Petrelli & Figà-Talamanca, 2001; Sallmén et al., 2003; Idrovo et al., 2005; Bretveld et al., 2006; Lauria et al., 2006; Bretveld et al., 2008; Joffe et al., 2008).

There have been some reports in the literature of adverse reproductive outcomes associated with pesticide use, most of which are described in more detail in a recent review (Wigle et al., 2008). Arbuckle et al. (2001) observed a rise in the risk of early abortion when preconception self-reported exposures to phenoxyacetic acid herbicides were present (odds ratio [OR] = 1.5, CI_{95%} 1.1–2.1; positive effect if greater than 1) and for late abortions, self-reported preconception exposure to glyphosate (OR = 1.7, CI_{95%} 1–2.9) was associated with higher risks. In another study, Curtis et al. (1999) reported a positive association (decrease in fecundability of 20% or more) measured through the outcome, TTP, when both spouses reported exposure to pesticide activities, with 5 of 13 pesticides categories (dicamba, glyphosate, phenoxy herbicides, organophosphorus insecticides, and thiocarbamates). Garry et al. (2002), studying pesticide applicators in Minnesota through a cross-sectional study of 695 workers and 1532 children (offspring), observed that self-reported use of the herbicide glyphosate yielded an OR of 3.6 (CI_{95%} 1.3–9.6) in relation to attention deficit disorder/attention deficit-hyperactivity disorder (ADD/ADHD), and pointed out that herbicides applied in the spring might be a factor in the birth defects.

Our objective in the current study was to test for differences in TTP for first pregnancy among fertile women selected from five regions of Colombia with different use patterns of glyphosate. This study also took into account other known factors affecting fecundability. A priori, it was postulated that the use of glyphosate in aerial spraying programs for eradication of illicit crops might be associated with reduced fecundability, and, considering that there are no biomarkers for exposure to glyphosate, an ecological exposure index was chosen.

MATERIALS AND METHODS

Design and Population

Between August 2004 and February 2005, a cross-sectional study of first pregnancies was carried out among women based on residence in one of five different regions (departments) from

Colombia (Figure 1). All participants were informed about the objectives of the study, and invited to participate if their first pregnancy occurred during the last 5 yr (since November 1999) and they did not use contraceptives during the year prior to becoming pregnant. The latter was to reduce reporting bias because there is no accurate method to adjust for the effect of the use of contraception on fecundity (Tingen et al., 2004). Only data on first pregnancies were used, to reduce recall bias and other potential biases that are associated with subsequent pregnancies. Only one pregnancy was used to maintain outcome independence and minimize the effect of previous reproductive history (Olsen & Skov, 1993).

Two days of training were carried out for interviewers and supervisors to explain the objectives of the project and the questionnaire to be applied. All interviewers lived in the study area and were supervised by local epidemiologists who knew the study area and who were well known to the population. In each area, studies started at the closest household where water and sediment samples were taken as part of the assessment of aerially applied glyphosate (Solomon et al., 2007). From the first household, the interview team moved away (centrifugally), visiting house by house to identify women who met the inclusion criteria until the sample size (600 women in each zone) was achieved. Because field workers were well known by the population, there were no refusals to enter the study, except in Valle del Cauca, where 3% of identified women declined to enter the study, mainly because their husbands did not allow them to participate. There were some differences among the five study sites that required us to visit more households in some areas than in others. For example, in Boyacá and Nariño, women start families at an early age; thus, when asked about first pregnancy in the last 5 yr there were many who were in the appropriate age group but had their first pregnancy more than 5 yr previously and therefore did not meet the inclusion criteria. In Valle del Cauca, most women had taken oral contraceptives in the last year, an exclusion criterion for the study. The population of Valle is different because it is a more developed department, was one of the first departments (if not the first) where extended family planning was initiated in the 1960s, and many villages (*veredas*) needed to be visited in order to obtain the sample size.

All women responding to the oral invitation were interviewed in their homes. Those who were confirmed as meeting the inclusion criteria were informed about the objectives of the study. Care was taken to ensure participants that there would be no reprisal for participation or nonparticipation, and that the investigators guaranteed the privacy of the information collected. Each participant provided written informed consent, in keeping with ethical approval by the Ethics Review Board of the Fundación Santa Fé de Bogotá, Colombia. Of a total of 3005 women interviewed, 233 women were excluded without TTP data and 21 with TTP values greater than 60 mo. Hence, 2751 (91.6%) were included in the analyses. However, for the



FIG. 1. Location of the study areas in Colombia (departments).

multiple regression and the alternative models, a restricted analysis was conducted without the 159 women who reported consultation with a physician because of fertility problems. This removed potential bias that may have been introduced by those who suspected themselves to be subfertile (Tingen et al., 2004; Idrovo et al., 2005; Joffe et al., 2005)

Exposure Assessment

As exposure could not be measured directly, an ecological design was used in which five different regions in the country, with different levels of exposure, were selected according to agricultural practices and presence or not of the aerial spray program for eradication of illicit crops with glyphosate. Table 1 shows the characteristics of the study areas.

Outcome Measurement

Valid data on TTP can be derived retrospectively, with a recall time of 14 yr or more (Joffe et al., 1995). A modified version of the key question from the questionnaire of Baird et al. (1986) was used to elicit TTP: "How many months were

you having sexual intercourse before you became pregnant for the first time?" The questionnaire was field tested in the five different regions to ensure the question was clearly understood in all areas since the departments are far from each other and there are subtle differences in understanding some terms. TTP was defined as duration in months, not divided by menstrual cycle duration in days, because women are more able to recall time in months than in cycles (Joffe, 1997). In this case, months and cycles were treated as equivalents.

Potential Confounders

During the interview, participants also provided information on potential confounders, including age at which the woman started trying to become pregnant, age at first pregnancy, and current age; relationship with partner; work history and gynecologic and medical history prior to first pregnancy; x-ray exposure in the year prior to conception; body image perception prior to conception as a proxy for body mass index (Singh, 1994; Madrigal-Fritsch et al., 1999; Romieu et al., 2004); and lifestyle practices in the year prior to conception, such as

TABLE 1
Geographic, Population, Agriculture, and Pesticide Use Characteristics in the Study Regions

Department	Description of the study area	Most common crops	Usual level of pesticide use	Glyphosate aerial spray program
Boyacá	Boyacá is located 130 km northwest of Bogotá (Figure 1), and the west zone is at 850 m a.s.l. with an annual mean temperature of 24°C and rainfall of 2250 mm per year. Ten out of the 132 municipalities belong to this zone and 5 were selected for the study. Boyacá has 1,404,309 inhabitants. 36,136 of which reside in the municipalities selected. Thirty percent are women between 15 and 49 yr of age. The inhabitants of this department are mainly mestizos.	Vegetables, potato, maize, barley, sugar cane, wheat, plantain, and some fruits are the main crops in the area. Illicit crops have been grown since 1992	Herbicides, fungicides, insecticides, and rodenticides are used in the study area. Glyphosate is used as herbicide.	None (manual eradication of illicit crops)
Nariño	Tumaco (located in Nariño, Figure 1) is one of the largest municipalities in the country (3760 km ²), located at 2 m a.s.l., has an annual mean temperature of 28°C and rainfall of 2,531 mm. It is located at the southwest corner of the country and border Ecuador to the south. Forty-nine percent of the 162,606 inhabitants live in rural areas. The projected number of women in fertile age (15–49 yr) for 2004 was 36,386. The population of Tumaco is largely of African origin.	Agriculture makes up 80% of the economic activities of the population. Main crops are oil (African) palm, cacao, plantain, and coconut. Forestry is another important source of income, as well as fish and aquaculture. Illicit crops have been grown in the area during the last 10 yr and currently represent 10–12% of the production of drugs in Colombia.	Herbicides, fungicides, and insecticides are used in the study area but people report very low use of these compounds.	Aerial spraying of glyphosate has been conducted since 1999 and Tumaco accounts for 25–30% of the total spraying in the department of Nariño.
Putumayo	The project was carried out in the municipality of Puerto Asis located at 260 m a.s.l. with an annual mean temperature 27°C. The area has 68,112 inhabitants, of whom 57.7% live in rural areas. Twenty percent of the total inhabitants are women of fertile age. The population of Putumayo and in the study area is mainly mestizo but people of Indian descent are found in this department.	Small crops of maize, plantain, and cassava are grown for local use.	Herbicides, fungicides, and insecticides are used in the study area. Low use of these compounds is reported by local inhabitants.	In 2003 and 2004, 15–24% of the aerial spraying of coca in Putumayo occurred in this municipality, indicating extensive spraying in the study area.
Sierra Nevada de Santa Marta	The National Park Sierra Nevada de Santa Marta is in the northern part of Colombia and has the highest peaks in the country, reaching more than 5000 m a.s.l. The selected area for the study is at the foothills of the Sierra at 700 m a.s.l. and rainfall of 3000 mm per year. The rural population of Santa Marta has 13,000 women 15–49 yr of age but only one village, where organic crops are cultured, was chosen for the study. The ethnicity of the population of the study area is mestizo but Indian heritage is less prevalent than in Putumayo or Boyacá.	Coffee plantations are the main crop in the study area of this region. Banana, peanuts, oranges, plantain, a type of sweet potato, and cacao, are also grown in the area.	No pesticide use for coffee plantation and other crops. Instead, native plants and biological control of pests are utilized.	None
Valle del Cauca	The study was carried out in the municipalities of Candelaria and Cerrito and the “ <i>corregimiento</i> ” (not large enough to be a municipality) of Rozo. Located at 1000 m a.s.l. with an annual mean temperature of 24°C. Approximately 13% of the population lives in rural areas. The population in the area includes mestizos as well as people of African origin.	Main crop in Valle del Cauca and in the study area is sugar cane. Coffee, plantain, and banana are also grown to a lesser extent.	Substantial herbicide use in sugar cane including glyphosate.	Aerial spraying of glyphosate for cane maturation 2 wk before harvesting.

smoking, drug, coffee, and alcohol consumption. Data on life style practices and work status for the father were also collected. A variable for self-perception of pollution of water was included, as well as one related to the source of water consumption in the current domicile.

Statistical Analysis

For analysis purposes, if TTP was reported as zero months (or "unexpected"), the answer was interpreted as 1 mo. Cut points for categorization of continuous variables were set as follows: age at time of interview at ≤ 25 yr; age when attempting to get pregnant and age when first becoming pregnant was set at ≤ 20 yr. For each exposure and potential confounder variable, analysis of variance (ANOVA) of mean TTP was conducted.

Among the 2592 women, 2477 pregnancies and 12,393 months (11,033 for final model) were included in multivariate models. Each month was classified according to the relevant exposure and confounder variables and an indicator variable was generated for every month, giving information on whether the cycle under this exposure resulted in a pregnancy or not. Fecundability odd ratios (fOR) were calculated with 95% confidence intervals (95% CI) using a discrete time analogue of Cox's proportional hazard model (Baird et al., 1986; Curtis et al., 1999; Zhou & Weinberg, 1999). Because TTP was assessed for a period of 12 mo, a separate censor variable was introduced if a woman took >12 mo to conceive. A value of 0 (noncensored) was used if TTP was ≤ 12 mo and 1 if TTP was >12 mo. fOR below unity indicate subfertility. All analyses were performed using Stata 7.0 (Stata Corporation, College Station, TX) with macros developed by Dinno (2002).

The initial saturated multivariate model included all variables significant on bivariate analysis ($p < .10$) and variables of prime biological importance (age at time of trying to become pregnant). Several goodness-of-fit statistics for logistic regression were checked: Pearson chi-square, deviance, and Hosmer-Lemeshow statistics (Hosmer & Lemeshow, 1989). The final model consisted of only those variables that contributed to the explanatory value of the model at a .05 level of significance (coefficient of determination). Collinearity was tested with VIF (variance inflation factor). The assumption that the fecundability odds ratio was constant across time (Weinberg & Wilcox, 1998) was tested graphically and by including an interaction term between months to pregnancy and exposure or confounder variables in the final model. The latter were not significant, implying that the proportional assumption was not violated. Finally, to evaluate a possible selection bias based on wantedness, the analyses were repeated excluding the pregnancies occurring in the first month (Weinberg et al., 1994). No significant changes in the final model were observed.

An alternative model without perfect fitting is presented for the sake of research interest, even though it had some marginal variables (p values $>.05$).

RESULTS

TTP showed large differences in different regions (Table 2). The Department of Valle del Cauca displayed a low percentage for the first month and Boyacá and Nariño were exceptionally high for the twelfth month (Figure 2).

Participating women were generally young (mean and median age 21 yr, range 15–48 yr, but there was one of 54 yr of age) and had completed at least some secondary education (Table 3). The vast majority had regular menstrual cycles (96.7%); a substantial proportion had irregular partner relationships. Most became pregnant first at young ages (73.6% at 20 yr of age or less). During the year before first pregnancy (YBP), most were free of illness (84.3%), had not had x-rays (95.4%), and did not smoke tobacco (95.1%). Alcohol and coffee consumption were 51.8% and 80.3%, respectively.

In the crude analyses (Table 3), longer TTP was associated with region, older maternal age, ethnic group, irregular menstrual cycles, and irregular partner relationship. Previous visits to physician for problems related with fertility, x-rays taken in the year before pregnancy (YBP), and coffee consumption in the YBP were associated with longer TTP. A significant trend between coffee consumption and longer TTP was observed. Maternal overweight showed a borderline significant association with a longer TTP.

The majority of women were housekeepers at the time they become pregnant. A tendency to longer TTP was observed among those engaged in some waged work and with higher education. Paternal unemployment or self work, were associated with longer TTP. No other paternal data were related to the outcome.

Self-perception about bad quality of water was associated with longer TTP, and all sources of water presented risk when they were compared with pure water ("nacimiento"), except some few cases that used carried water ("carro-tanque").

TABLE 2
Time to Pregnancy and Percentage of Pregnancy
by Month in the Study Regions

Months	Regions					Total
	Boyacá	Nariño	Sierra Nevada de Santa Marta	Putumayo	Valle del Cauca	
1	69.2	21.2	25.5	49.4	17.0	36.8
3	82.5	62.9	52.9	56.1	28.7	57
6	88	94.8	72.1	74.9	45.2	75.2
12	96.9	99.3	87.3	89	73.5	89.4
MTTP ^a	3	3.3	8.6	6.4	14	7
MTTP ^b	3	3.3	7.1	6	12.6	6.3

^aMTTP, mean time to pregnancy in months.

^bCensored to 60 mo (see text).

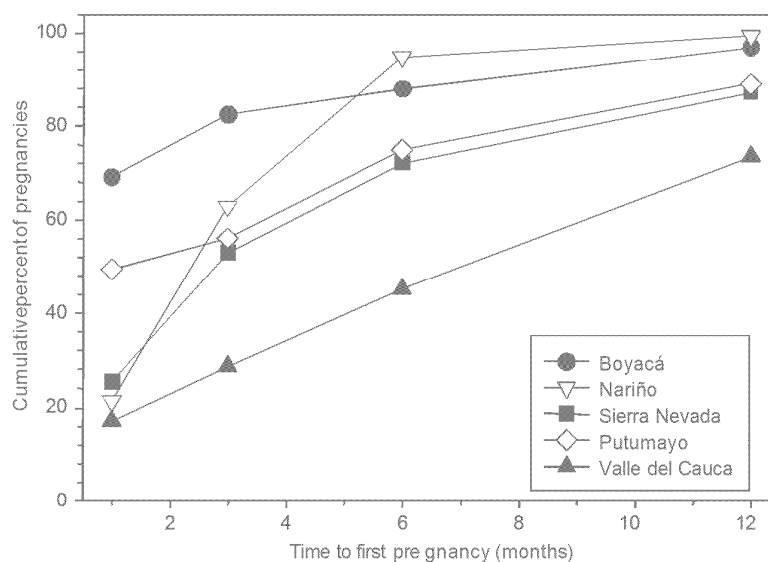


FIG. 2. Unadjusted cumulative percentage of pregnancies over time for the five study regions in Colombia.

In the final multivariate model (Table 4), the main predictor was region adjusted by irregular relationship with partner and maternal age at first pregnancy. Boyacá displayed minimal risk and was used as the reference. Nariño, Sierra Nevada de Santa Marta, and Putumayo showed higher risk, with the highest risk in Valle del Cauca. Goodness-of-fit statistics for the final model were optimal when adjustment for maternal age when the first pregnancy had occurred was carried out. Table 4 shows the analysis without including 159 women who reported visiting a physician because of fertility problems. In the crude analysis, irregular cycles and medication for this purpose were associated with longer TTP, but when potentially subfertile couples were excluded, these two variables were no longer included in the final model. Age at first pregnancy and irregular relationship remained in the model after excluding those with fertility problems. Table 5 shows that coffee consumption and perception of contamination of water, although no longer significant, were borderline. When categorized in number of cups, coffee consumption still showed a positive trend; the greater the number of cups, the longer was the TTP.

An alternative model is presented in Table 5 because that model includes variables such as coffee consumption and water pollution with marginal statistical significance but with strong biological and environmental significance.

DISCUSSION

This was the first study performed in Colombia with the objective of assessing whether an association existed between use of aerially applied glyphosate for eradication of illicit crops and subchronic effects on reproduction, such as TTP. A major

problem in many epidemiological studies is the lack of appropriate exposure data based on actual measurements (Arbuckle et al., 2002; Harris et al., 2002; Coble et al., 2005; Ritter et al., 2006; Firth et al., 2007). In most cases, exposures are approximated through questionnaires, geographical regions, type of crop, season of application, chemical group, or classification according to mode of action (herbicides, insecticides, fungicides, etc). This is done because most pesticides lack a persistent biomarker, which prevents a measurement-based characterization of exposure for the majority of the pesticide products, including glyphosate (Acquavella et al., 2004).

For this reason, the acute effects of this herbicide are the most extensively documented (Acquavella et al., 1999) with predominant manifestations being eye irritation and other temporary dermal effects. Whether pneumonitis occurs is controversial (Pushnoy et al., 1998), and fatal cases have been recorded only with accidents or when glyphosate was ingested with the purpose of committing suicide (Williams et al., 2000). Some cases of Parkinson's disease have been associated with acute intoxication with glyphosate (Barbosa et al., 2001), but the small number of cases and lack of laboratory animal analogies do not allow assignment of causality.

Some authors have made efforts to identify the compounds used by study subjects. Several studies on different populations that specifically addressed the use of glyphosate were found and published since the last major reviews (Williams et al., 2000; Solomon et al., 2007). Studies related to cancer and to adverse reproductive and developmental effects reported equivocal and unclear relationships between glyphosate use and some reproductive outcomes (Curtis et al., 1999; Arbuckle et al., 2001; Garry et al., 2002; De Roos et al., 2005).

TABLE 3
Mean Time to Pregnancy (Without Censoring) and Crude Fecundability Odds Ratio (fORc)
Analyzed by Different Sociodemographic Characteristics

Variable	<i>n</i>	Time to pregnancy (mo), <i>X</i> (SD) ^a	fORc (CI _{95%}) ^b	<i>p</i>
Region				
Boyacá	582	3 (4.7)	1	—
Nariño	552	3.3 (3.3)	0.72 (0.62, 0.83)	<.01
Sierra Nevada de Santa Marta	551	7.1 (10.3)	0.41 (0.35, 0.48)	<.01
Putumayo	535	6 (8.3)	0.44 (0.38, 0.51)	<.01
Valle del Cauca	531	12.6 (13.5)	0.2 (0.17, 0.24)	<.01
Maternal age (yr)				
≤25	2356	5.7 (8.2)	1	—
>25	395	10 (14)	0.64 (0.56, 0.73)	<.01
Age to first pregnancy (yr)				
≤20	2026	5.5 (8)	1	—
>20	725	8.6 (12.3)	0.69 (0.62, 0.76)	<.01
Age at start TTP study period (yr)				
≤20	2094	6.2 (9)	1	—
>20	657	6.8 (10.5)	0.98 (0.88, 1.1)	.69
Ethnic group				
Mestizo	2121	6.5 (9.6)	1	—
Negro	508	6.3 (9.6)	1. (0.9, 1.14)	.83
Indígena	49	3.7 (4.1)	1.37 (0.9, 1.94)	.08
Zambo	41	3.6 (2.6)	1.38 (0.95, 2.01)	.09
Mulato	32	3.3 (2.6)	1.6 (1.05, 2.51)	.03
Grouped ethnic group				
Mestizo and Negro	2629	6.45 (9.6)	0.7 (0.56, 0.87)	<.01
Indígena, Zambo, and Mulato	122	3.54 (3.2)	1	—
Education				
None	42	4.9 (9.1)	1	—
Incomplete elementary school	582	4.6 (7.1)	0.93 (0.63, 1.39)	.74
Complete elementary school	526	5.6 (7.7)	0.76 (0.51, 1.13)	.17
Incomplete high school	459	7 (10.3)	0.66 (0.45, 0.98)	.04
Complete high school	130	9.7 (12.5)	0.47 (0.31, 0.73)	<.01
Zone				
Urban	5	15.2 (18.6)	—	—
Rural	2743	6.3 (9.4)	—	—
Marital status ^c				
Common law	1010	5.1 (8.3)	1	—
Not common law	1741	7 (9.9)	0.71 (0.64, 0.78)	<.01
Socioeconomic status ^{c,d}				
0	38	4.8 (5.8)	1.1 (0.75, 1.63)	.62
1	2013	6.4 (9.4)	1	—
≥2	493	6.6 (10.1)	1.03 (0.91, 1.17)	.60
Nutritional status ^e				
Low weight	111	7.1 (10.9)	0.91 (0.72, 1.15)	.42
Normal weight	2453	6.2 (9.3)	1	—
Overweight	184	7.2 (10.0)	0.83 (0.69, 1.00)	.04
Maternal work ^c				
Administrative, teacher, or student	678	6 (8.6)	0.99 (0.89, 1.11)	.92

(Continued)

TABLE 3
(Continued)

Variable	<i>n</i>	Time to pregnancy(mo), <i>X</i> (SD) ^a	fORc (CI _{95%}) ^b	<i>p</i>
Home, no work, housekeeper	1631	6 (9.2)	1	—
Community mother, mining, various, other, occasional	229	8.7 (11.9)	0.7 (0.59, 0.83)	<.01
Health worker, independent worker, seller	126	8.7 (12.3)	0.74 (0.59, 0.93)	.01
Agriculture and floriculture	86	4.2 (4.6)	1.14 (0.87, 1.48)	.34
Maternal work in cocaine ^c				
No	2743	6.3 (9.4)	1	—
Yes	8	7.6 (7.4)	0.67 (0.29, 1.54)	.35
Menarche age (yr)				
<12	1031	6.6 (10)	1	—
13	802	5.8 (8.6)	1.06 (0.94, 1.19)	.32
14	523	6.4 (9.3)	1.02 (0.89, 1.16)	.81
15	392	6.6 (9.4)	0.94 (0.82, 1.09)	.43
Menstrual cycle				
Regular	2612	6.2 (9.3)	1	—
Irregular	88	9.5 (12.3)	0.64 (0.49, 0.84)	<.01
Previous consultation for pregnancy problems				
No	2592	5.8 (8.7)	1	—
Yes	159	15.2 (14.4)	0.33 (0.27, 0.41)	<.01
Smoking ^c				
No	2616	6.3 (9.5)	1	—
Yes	135	6.2 (7.9)	0.95 (0.77, 1.17)	.63
Alcohol consumption ^c				
No	1325	6.2 (9.4)	1	—
Yes	1425	6.3 (9.4)	0.97 (0.88, 1.06)	.52
Coffee consumption ^c				
No	543	5.3 (8.1)	1	—
Yes	2208	6.6 (9.7)	0.81 (0.72, 0.91)	<.01
Number of coffee cups/day ^c **				
0	543	5.3 (8.1)	1	—
1 to 3	1916	6.4 (9.5)	0.83 (0.73, 0.93)	<.01
≥4	292	7.4 (10.7)	0.73 (0.61, 0.87)	<.01
X-rays ^c				
No	2616	6.2 (9.2)	1	—
Yes	125	9.4 (12.5)	0.67 (0.54, 0.84)	<.01
Any kind of illness ^c				
No	2316	6.3 (9.3)	1	—
Yes	432	6.4 (9.7)	0.97 (0.86, 1.10)	.68
STD ^{c,e}				
No	2717	6.3 (9.3)	1	—
Yes	27	7.3 (10.4)	0.84 (0.52, 1.38)	.50
Medication for regularizing menses ^c				
No	2721	6.3 (9.4)	1	—
Yes	30	11.2 (10)	0.45 (0.28, 0.71)	<.01
Medication for “sugar in blood” ^c				
No	2735	6.3 (9.4)	1	—

(Continued)

TABLE 3
(Continued)

Variable	<i>n</i>	Time to pregnancy(mo), <i>X</i> (SD) ^a	fORc (CI _{95%}) ^b	<i>p</i>
Yes	16	7.6 (6.5)	0.7 (0.39, 1.24)	.22
Other medications ^c				
No	2027	6.8 (9.7)	1	—
Yes	703	5 (8.3)	1.36 (1.22, 1.51)	<.01
Paternal work ^c				
Administrative or student	160	6.3 (8.2)	0.86 (0.7, 1.05)	.14
No work, occasional	212	8.4 (12.5)	0.74 (0.61, 0.88)	<.01
Carpenter, driver, construction, mining, mechanic, industrial timbering	507	5.7 (9)	1.02 (0.89, 1.16)	.80
Other, health worker, independent worker, vendor	713	7.7 (10.8)	0.75 (0.67, 0.84)	<.01
Agriculture, floriculture, livestock	1157	5.4 (7.9)	1	—
Paternal work in cocaine ^c				
No	2457	6.4 (9.6)	1	—
Yes	292	5.5 (7.8)	1.07 (0.92, 1.24)	.40
Any disease of the father ^c				
No	2398	6.4 (9.5)	1	—
Yes	248	6.8 (9.4)	0.93 (0.80, 1.1)	.41
STD of the father ^{c,f}				
No	2608	6.4 (9.5)	1	—
Yes	37	6.1 (8.2)	0.99 (0.66, 1.47)	.94
Paternal alcohol consumption ^c				
No	1325	6.2 (9.4)	1	—
Yes	1425	6.4 (9.4)	0.97 (0.88, 1.06)	.52
Paternal smoking ^c				
No	2143	6.2 (9.3)	1	—
Yes	538	6.9 (10)	0.9 (0.80, 1.01)	.08
Paternal use of psychotropic drugs ^c				
Yes	2619	6.4 (9.5)	1	—
No	54	4.5 (5.7)	1.23 (0.88, 1.72)	.22
Perceived contamination of water				
No	1218	6 (9.2)	1	—
Yes	1533	6.6 (9.5)	0.9 (0.82, 0.98)	.02
Source of drinking water				
Municipal tap water	598	7.2 (11)	1.13 (1, 1.28)	.05
Rain water	65	5.3 (8)	1.38 (1.02, 1.88)	.04
Stream, ravine, or creek	257	6.1 (10.2)	1.36 (1.14, 1.61)	<.01
“Carried water”	10	14.8 (16.5)	0.44 (0.19, 1.05)	.07
“Pure water”	311	3.7 (5.7)	2.03 (1.73, 2.39)	<.01
Deep well	1040	7.4 (10)	1	—
River	470	4.6 (6.5)	1.52 (1.33, 1.74)	<.01

**There is a significant trend when $p < .05$.^aMean and standard deviation.^bCrude fecundability odds ratio; 95% confidence interval.^cDuring the year prior to pregnancy.^dThe population is classified in 6 socioeconomic strata, from 1 being the lowest to 6 the highest. A zero indicates extreme poverty.^eBased on self-reporting images scaled from 1 to 9. Low weight 1 to 4, normal 5 to 7, overweight 8 and 9 (BMI ≥ 25) (Madrigal-Fritsch et al., 1999).^fSTD, sexually transmitted disease.

TABLE 4

Causes of Fecundability Adjusted^a for the Relationship Between Time to Pregnancy (TTP) and Region^b

Variable	fRMA ^c	EE ^d	IC _{95%} ^e	<i>p</i>
Region ^f				
Nariño	0.53	0.044	0.45, 0.63	<.01
Sierra Nevada	0.36	0.030	0.30, 0.42	<.01
Putumayo	0.34	0.029	0.29, 0.41	<.01
Valle del Cauca	0.15	0.013	0.12, 0.18	<.01
Age at first pregnancy >20 yr ^g	0.81	0.048	0.72, 0.91	<.01
Irregular relationship with father ^h	0.76	0.041	0.68, 0.84	<.01

Note. *n* = 2592 mothers 11,270 cycles.

^aProportional risk model of Cox, modified after Dinno, (2002).

^bRestricted to those mothers who did not consult a physician regarding problems in conceiving.

^cfRMA Adjusted cause of fecundability.

^dStandard error.

^e95% Confidence interval.

^fCompared to Boyacá as reference.

^gCompared to ≤20 years as reference.

^hCompared to regular relationship as reference.

Other risk factors present in the rural and agricultural environment of the women studied and individual characteristics (genetic, for example) may be associated with TTP. Longer TTP were observed in some populations with higher physical activity (Florack et al., 1994) or psychological distress (Hjollund et al., 1999). Further, TTP may be influenced by knowledge and behavior, such as patterns of intercourse as well as biologic factors (Joffe et al., 2005), and these need to be considered as potential confounders (Tingen et al., 2004; Stanford & Dunson, 2007). The potential effect of these factors on TTP could not be isolated in this study, even though the fOR was adjusted for most known confounders and independent predictors.

As shown in Figure 2, there was no difference in cumulative TTP between Putumayo, where illicit crops were sprayed, and Sierra Nevada, where there was no herbicide use. In turn, the latter region showed lower cumulative percent pregnancies than Nariño, an eradication spray area, and Boyacá, where there is agricultural herbicide use but manual eradication of illicit crops. Although classification of exposure may be a source of bias in this type of study, no relationship between reduced fecundability in the studied regions and use of glyphosate specifically for spray eradication or use of pesticides in general can be established from our data. Prospective studies that prevent or reduce classification bias of exposures are recommended to further elucidate relationships between aerial spraying of glyphosate for eradication, agricultural pesticide use, and human health indicators.

TABLE 5

Causes of Fecundability Adjusted^a for the Relationship Between Time to Pregnancy (TTP) and Region^b Based on an Alternative Model

Variable	fRMA ^c	EE ^d	IC _{95%} ^e	<i>p</i>
Region ^f				
Nariño	0.56	0.048	0.47, 0.66	<.01
Sierra Nevada	0.36	0.031	0.31, 0.43	<.01
Putumayo	0.35	0.029	0.29, 0.41	<.01
Valle del Cauca	0.15	0.014	0.13, 0.18	<.01
Age at first pregnancy >20 yr ^g	0.81	0.048	0.73, 0.91	<.01
Irregular relationship ^h	0.76	0.041	0.68, 0.84	<.01
Consumption of coffee ⁱ				
Medium (1–3 cups per day)	0.91	0.059	0.81, 1.04	.15
High (4 and more cups per day)	0.84	0.083	0.69, 1.02	.08
Perception of contamination of water ^j	0.91	0.51	0.81, 1.01	.08

Note. *n* = 2592 mothers, 11,270 cycles.

^aProportional risk model of Cox, modified after Dinno (2002).

^bRestricted to those mothers who did not consult a physician regarding problems in conceiving.

^cfRMA Adjusted cause of fecundability.

^dStandard error.

^e95% Confidence interval.

^fCompared to Boyacá as reference.

^gCompared to ≤20 years as reference.

^hCompared to regular relationship as reference.

ⁱCompared to no consumption as reference.

^jCompared to no contamination as reference and based on self-perception and source of water normally consumed.

Pesticides in general are likely not the cause either, as large differences in TTP were observed between two regions of high to moderate pesticide use, Valle del Cauca and Boyacá. The observed ecological differences remain unexplained, but may be produced by varying exposures to environmental factors, history of contraceptive programs in the region, or psychological distress. Future studies examining these causes are needed.

Table 3 shows crude association between coffee consumption and longer TTP with a significant trend. This association is not significant in the adjusted model but the level of significance was borderline. Published results regarding coffee or caffeine consumption and TTP are not conclusive. Some studies showed no association (Joesoef et al., 1990; Alderete et al., 1995), but other investigators found that coffee drinkers have a lower risk of pregnancy (Wilcox et al., 1988; Christianson et al., 1989; Williams et al., 1989; Hatch & Bracken, 1993; Curtis et al., 1997). This relationship needs to be further investigated.

Distribution of pregnancies in relation with months in different regions showed great differences (Table 2). In a previous study in Colombia (Idrovo et al., 2005), the percentage for first month was close to 30%, which is lower than more than 40% reported from a Danish study (Joffe et al., 2005). In our study, the region of Valle del Cauca showed a low percentage and Boyacá exceptionally high for first and twelfth months (Figure 2). The mean for 12 mo in developed countries is between 85 and 90%. These results are consistent with the National Survey of Demography and Health (Ojeda et al., 2005) that showed Boyacá as the Department with the lowest proportion of women who reported fertility problems (4.2%). Valle del Cauca (11.2%) and Magdalena (16.1%), where Sierra Nevada is located, were above the national average (10.6%).

A retrospective assessment of TTP as an outcome variable was conducted to evaluate ecological exposure to glyphosate. Although it is widely recognized that retrospective studies for TTP can be carried out, they are prone to some biases that need to be taken into account in the interpretation of our results. Difference in sexual behavior between exposed and nonexposed subjects, particularly in frequency of intercourse, has been pointed out as source of bias (Tingen et al., 2004; Stanford & Dunson, 2007). Women of reproductive age differed in reporting intercourse in the last 4 wk, from 48.8% in Boyacá and neighboring departments to 53.8% in the Pacific region where Tumaco (Nariño) is located (Ojeda et al., 2005). Couples who had not used contraception in the last year were included and, in the multivariate analysis, those who had had consultation because of fertility problems were excluded. These two criteria excluded those who may have become pregnant while using contraception (highly fecund couples) and subfertile couples (Bonde et al., 2006). Studies also evaluated whether there were other sources of bias such as pregnancy recognition (Joffe et al., 2005) by asking whether a miscarriage occurred, and thus it was possible to control for this variable. However, biological factors such as age at first pregnancy and use of contraception in the past were taken into account as these appear to be more important than lifestyle factors in assessing TTP (Axmon et al., 2006).

Classification of exposure was by location of residence. Nonexposed participants were those who lived in the region where organic crops were produced and who, in addition, did not report any use of pesticides in the interview. In the other four departments, there was exposure not only to glyphosate, but also to other herbicides and pesticides. Although place of residence is not an accurate surrogate for exposure to pesticides and may generate misclassification (Arbuckle et al., 2004), this ecological assessment is useful to explore, at the population level, whether an association exists between the putative exposure (aerial spraying of glyphosate) and outcome (Ritter et al., 2006). However, in this study, aerial spraying of glyphosate was not consistently associated with delayed time to pregnancy.

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To: Deener, Kathleen[Deener.Kathleen@epa.gov]
From: Gwinn, Maureen
Sent: Fri 5/6/2016 5:34:43 PM
Subject: RE: sorry to bother you again..

Let me get Jackie – I'll let you know what I find out!

Maureen R. Gwinn, PhD DABT ATS

Special Assistant/IOAA

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From: Deener, Kathleen
Sent: Friday, May 06, 2016 1:34 PM
To: Gwinn, Maureen <gwinn.maureen@epa.gov>
Subject: RE: sorry to bother you again..

I'm fuzzy on those details, too. I'll see what I can find out. You could reach out to Andrea Mojica, too. Or Jackie McQueen in OSP probably has a good understanding of things.

Kacee Deener, MPH

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deener.kathleen@epa.gov

From: Gwinn, Maureen
Sent: Friday, May 06, 2016 1:15 PM
To: Deener, Kathleen <Deener.Kathleen@epa.gov>

Subject: sorry to bother you again..

Trying to piece together the role of the CARC in OPP – seems like it can be the final word on assessments, but some may go further and be sent to the SAP (not sure if glyphosate was slated to – I don't see anything on the SAP website). Does that sound right to you? Bob wanted to know for today's meeting, whatever the topic is...

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Glyphosate and aminomethylphosphonic acid are not detectable in human milk^{1,2}

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ABSTRACT

Background: Although animal studies have shown that exposure to glyphosate (a commonly used herbicide) does not result in glyphosate bioaccumulation in tissues, to our knowledge there are no published data on whether it is detectable in human milk and therefore consumed by breastfed infants.

Objective: We sought to determine whether glyphosate and its metabolite aminomethylphosphonic acid (AMPA) could be detected in milk and urine produced by lactating women and, if so, to quantify typical consumption by breastfed infants.

Design: We collected milk (n = 41) and urine (n = 40) samples from healthy lactating women living in and around Moscow, Idaho and Pullman, Washington. Milk and urine samples were analyzed for glyphosate and AMPA with the use of highly sensitive liquid chromatography–tandem mass spectrometry methods validated for and optimized to each sample matrix.

Results: Our milk assay, which was sensitive down to 1 mg/L for both analytes, detected neither glyphosate nor AMPA in any milk sample. Mean \pm SD glyphosate and AMPA concentrations in urine were 0.28 \pm 0.38 and 0.30 \pm 0.33 mg/L, respectively. Because of the complex nature of milk matrixes, these samples required more dilution before analysis than did urine, thus decreasing the sensitivity of the assay in milk compared with urine. No difference was found in urine glyphosate and AMPA concentrations between subjects consuming organic compared with conventionally grown foods or between women living on or near a farm/ranch and those living in an urban or suburban nonfarming area.

Conclusions: Our data provide evidence that glyphosate and AMPA are not detectable in milk produced by women living in this region of the US Pacific Northwest. By extension, our results therefore suggest that dietary glyphosate exposure is not a health concern for breastfed infants. This study was registered at clinicaltrials.gov as NCT02670278. Am J Clin Nutr doi: 10.3945/ajcn.115.126854.

Keywords: AMPA, glyphosate, human milk, lactation, organic food, aminomethylphosphonic acid, breastfeeding, environmental contaminants, pesticide

INTRODUCTION

Glyphosate (N-phosphonomethyl glycine), a widely used herbicide patented as a phytotoxicant in 1974 (1), functions by

blocking the activity of 5-enolpyruvylshikimate-3-phosphate synthase (Enzyme Commission number 2.5.1.19), an enzyme required for the synthesis of tryptophan, phenylalanine, and tyrosine in plants and some microorganisms (2–5). Because these amino acids are not synthesized by humans, glyphosate would not be expected to have a physiological effect. Indeed, the human genome does not encode for 5-enolpyruvylshikimate-3-phosphate synthase, and a large body of epidemiologic and experimental literature supports the safety of glyphosate in mammals (5, 6). In addition, neither glyphosate nor its metabolite aminomethylphosphonic acid (AMPA)⁸ seem to bioaccumulate in animal tissues (7–9). In addition, most scientific evidence does not support contentions that glyphosate may cause cancer in humans, as recently concluded after a lengthy review by the European Food Standards Authority (10). The US Environmental Protection Agency (EPA) has authorized the use of glyphosate as an herbicide in noncrop and industrial areas since 1974 and in agriculture since 1976 (11). The safety of glyphosate use as an herbicide is periodically re-evaluated, with the last federal review completed in 1993 (12).

Despite its long-standing track record for safety, decades of research have resulted in a vast body of literature related to the clearance and disposition of ingested glyphosate. Studies in humans show that ~20% of diet-derived glyphosate is absorbed from the gastrointestinal tract, with the remaining ~80% excreted in the feces (13, 14), and studies conducted with rats suggest that nearly all absorbed glyphosate is rapidly excreted

¹ The authors reported no funding received for this study. This is a free access article, distributed under terms (<http://www.nutrition.org/publications/guidelines-and-policies/license/>) that permit unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

² Supplemental Figure 1 and Supplemental Table 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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⁸ Abbreviations used: AMPA, aminomethylphosphonic acid; EPA, US Environmental Protection Agency; LOD, limit of detection; LOQ, limit of quantification; RfD, reference dose.

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unchanged in urine (9, 15–18); little, if any, is metabolized to and excreted as AMPA. In fact, most AMPA in urine is thought to be the result of either the consumption of plants that have metabolized glyphosate into AMPA (12, 18, 19) and/or exposure to phosphonates found in detergents (20, 21).

Several studies have also investigated urine glyphosate concentrations of humans exposed to glyphosate via diet and other environmental sources (14, 22–25). These studies have consistently documented urine glyphosate concentrations of w 1–3 mg/L (in ppb), with the highest value being 233 mg/L (24). Curwin et al. (26) also reported urine glyphosate concentrations in 116 children living in “farm” and “nonfarm” households. Most samples (84%) had detectable concentrations with values similar to those reported in adults. There was no difference in urine glyphosate concentrations between children living in farm and nonfarm households (27). It is noteworthy that all measured urine glyphosate concentrations to date, even the highest, have not warranted a legitimate health concern based on the European Food Safety Authority’s allowable daily intakes and allowable operator exposure concentrations (14).

Of particular interest to our research group is the potential glyphosate exposure of infants during breastfeeding. Because there have been to our knowledge no studies published in peer-reviewed journals reporting glyphosate concentrations in human milk, this study (NCT02670278) was undertaken primarily to document typical glyphosate and AMPA concentrations in milk produced by lactating women living in the US Pacific Northwest—a highly productive agricultural region in which glyphosate-containing herbicides are routinely used (27). Maternal urine samples were also collected and analyzed. We hypothesized that concentrations of glyphosate and AMPA in milk and urine would be low, if even detectable. Important to testing this hypothesis was the use of newly optimized, matrix-specific assays with high sensitivities and specificities for the analytes (28).

METHODS

Human subjects

All procedures used in this study were approved by the Washington State University Institutional Review Board, and informed consent was obtained from each subject. A total of 41 healthy lactating women living in and around Pullman, Washington, and Moscow, Idaho, were included in the study, which was part of a larger investigation of international variation in human milk oligosaccharides and bacterial taxa as they relate to environmental exposure and sociocultural practices. To be eligible for participation, women had to be 1–3 mo postpartum, breastfeeding and/or pumping milk ≥ 5 times/d, and aged ≥ 18 y. Because we wanted to limit our subjects to healthy women who were nursing healthy infants, exclusion criteria included current breast infection, use of antibiotics in the previous 30 d, and having an infant with signs or symptoms of illness in the previous 7 d. Subjects completed a brief survey to document basic health and demographic variables, and body weight and height were measured at enrollment, which spanned from May 2014 through March 2015. All but 1 subject also completed a 5-question survey documenting potential glyphosate exposure from the environment and diet (Supplemental Figure 1).

Milk and urine collection and preservation

Milk and urine were collected between 0700 and 1100. After cleaning the breast (a step necessary to meet the needs of the larger, overarching project), w 30 mL milk was collected with the use of a Medela Symphony hospital-grade electric breast pump into a Medela Symphony single-use sterile collection container, immediately placed in ice, separated into aliquots while fresh, and then frozen at -20°C until analysis. A midstream urine sample was collected into a single-use sterile collection container. The sample container was immediately placed in ice, and urine was separated into aliquots and frozen at -20°C until analysis. One subject failed to provide a urine sample.

Glyphosate and AMPA analyses

Milk and urine samples were analyzed for glyphosate and AMPA at Monsanto with the use of liquid chromatography–tandem mass spectrometry methods optimized for and validated in each sample matrix (28). A Shimadzu Prominence 20A HPLC system coupled to an AB Sciex API 5500 triple-quadrupole mass spectrometer was used for analysis. Glyphosate and AMPA were quantitated with the use of multiple reaction monitoring. Two precursor-product ion transitions for each analyte and stable isotope labeled internal standard for each analyte were used to ensure the selectivity of the methods. Although 2 quantitative precursor-product ion transitions were monitored, the results were reported with the use of the most sensitive transition for each analyte. The assay was validated separately for milk and urine. Limits of detection (LODs) and quantification (LOQs) for glyphosate in milk were 1.0 and 10.0 mg/L, respectively; those for urine were 0.02 and 0.10 mg/L, respectively. LODs and LOQs for AMPA in milk were 1.0 and 10.0 mg/L, respectively; those for urine were 0.03 and 0.10 mg/L, respectively.

Glyphosate and AMPA concentrations in milk were independently confirmed by Covance with the use of the same liquid chromatography–tandem mass spectrometry method (28) with minor modifications, which included the use of an AB Sciex QTrap 5500 triple-quadrupole mass spectrometer. Because of differences in instrumentation, the LODs that used the more sensitive quantitative ion transitions were 6.0 and 9.0 mg/L for human milk glyphosate and AMPA, respectively, and the LOQ was 25.0 mg/L for both analytes.

It is noteworthy that duplicate aliquots (created from fresh milk at the time of collection) of each milk sample were sent directly, albeit separately, from Washington State University to Monsanto and Covance. Data generated by Covance were communicated directly to the principal investigators without prior disclosure to other coauthors.

Statistical analyses

All values for milk ($n = 41$) glyphosate and AMPA concentrations were below the LOD; thus, no statistical analyses on these data were warranted. For urine glyphosate and AMPA ($n = 40$), statistical analyses were conducted with the use of a generalized linear mixed model (SAS version 9.4; SAS Institute) assuming a Poisson distribution with a logarithmic link function. For concentrations less than the respective LOD values, one-half LOD (0.01 and 0.015 mg/L for glyphosate and AMPA, respectively) nominal values were used in the analyses. For

concentrations that fell between the LOD and LOQ, one-half LOQ (0.05 mg/L for both glyphosate and AMPA) nominal values were used in the analysis (29). All values presented represent means \pm 6 SDs.

RESULTS

Description of study population and glyphosate exposure

Basic demographic and anthropometric variables for the 41 study subjects are given in Table 1. Women were aged 29 \pm 5 y, 67 \pm 17 d postpartum, and had a BMI (kg/m²) of 26.8 \pm 8.6. Most (75%) lived in an urban or suburban nonfarming region of the Palouse (a geographical area encompassing southeastern Washington and northwestern Idaho), and most (58%) reported that they made no effort to eat foods characterized as organic, although they sometimes included them in their diets for convenience. Few subjects (15%) reported ever having personally mixed or used any type of weed killer; all but 1 of the women having reported ever doing so had mixed or used a weed killer containing glyphosate. In general, subjects were highly educated Caucasian women who participated in the study during either the summer or winter months.

Glyphosate and AMPA concentrations in milk

A summary of our findings concerning milk glyphosate and AMPA are found in Table 2. Regardless of whether the assays were conducted at Monsanto or Covance, none of the milk samples contained detectable amounts of either glyphosate or AMPA. As such, descriptive and statistical analyses were not warranted.

TABLE 1
Characteristics of the women participating in this study (n = 41)

Variable	Value
Age, y	29 \pm 5 ¹
Time postpartum, d	67 \pm 17
Parity, n	1.8 \pm 1.1
Body weight, kg	74.6 \pm 24.2
BMI, kg/m ²	26.8 \pm 8.6
Lived on or near a farm/ranch, ² %	25
Strictly or mainly organic food choices, ² %	42
Had at some time personally used or mixed any type of weed killer, ² %	15
Highest attained educational level, ² %	
High school	32
Undergraduate college degree	41
Graduate degree	27
Ethnicity, ² %	
Caucasian	93
African American	2
Latina	5
Season of sample collection, ² %	
Spring	12
Summer	46
Fall	17
Winter	27

¹Mean \pm 6 SD (all such values).

²Questionnaire data were missing for 1 woman; values represent those of the remaining 40 women.

TABLE 2

Mean glyphosate and AMPA concentrations (mg/L) in milk and urine produced by healthy women living in the US Pacific Northwest¹

Variable	Value
Milk (n = 41)	
Glyphosate ²	, LOD
AMPA ³	, LOD
Urine (n = 40)	
Glyphosate ⁴	0.28 \pm 0.38
AMPA ⁴	0.30 \pm 0.33

¹Values are means \pm 6 SDs. AMPA, aminomethylphosphonic acid; LOD, limit of detection; LOQ, limit of quantification.

²LOD = 1 and 6 mg/L when milk was analyzed at Monsanto and Covance, respectively; glyphosate could not be detected in any of the milk samples analyzed.

³LOD = 1 and 9 mg/L when milk was analyzed at Monsanto and Covance, respectively; AMPA could not be detected in any of the milk samples analyzed.

⁴Glyphosate and AMPA concentrations were less than the LOD or between the LOD and LOQ in 3 and 2 of the samples, respectively. For concentrations less than the LOD values, one-half LOD nominal values were used in the analyses; for those that fell between the LOD and LOQ, one-half LOQ nominal values were used. All analyses were conducted at Monsanto with an LOD and LOQ of 0.02 and 0.1 mg/L for glyphosate, respectively, and LOD and LOQ of 0.03 and 0.1 mg/L for AMPA, respectively.

Glyphosate and AMPA concentrations in urine

A summary of our findings concerning urine glyphosate and AMPA are found in Table 2 (raw data are available in Supplemental Table 1). Glyphosate was detectable in nearly all (n = 37) of the urine samples and was quantifiable in 29 of them. Glyphosate values ranged from below the LOD (, 0.02 mg/L) to 1.93 mg/L, with a mean of 0.28 \pm 0.38 mg/L. AMPA was also detectable in nearly all (n = 38) of the urine samples and quantifiable in 29 of them. Urine AMPA values ranged from below the LOD (, 0.03 mg/L) to 1.33 mg/L, with a mean of 0.30 \pm 0.33 mg/L. There were no significant effects of consuming organic compared with conventional foods or living on/near a farm compared with living in an urban/suburban region on concentrations of glyphosate in urine (P = 0.1870 and 0.8773, respectively) (Figure 1). Neither were there significant effects of consuming organic compared with conventional foods or living on/near a farm compared with living in an urban/suburban region on concentrations of AMPA in urine (P = 0.1414 and 0.2525, respectively) (Figure 2). Adjusting for potential covariates (age, time postpartum, BMI, parity) did not alter these conclusions. When raw, untransformed values were used in the analysis, there was a positive correlation (r = 0.57; P = 0.0001) between urinary glyphosate and AMPA concentrations. The strength of this association increased when log-transformed data were used (r = 0.68; P = 0.0001) (Figure 3).

DISCUSSION

The results herein provide evidence that the concentrations of glyphosate and AMPA in milk produced by healthy women are below the detection limits of available validated assays. In urine, glyphosate and AMPA were detectable in many samples, but concentrations were very low (, 0.02 to 1.93 and , 0.03 to 1.33 mg/L, respectively)—in fact, well below values reported in other

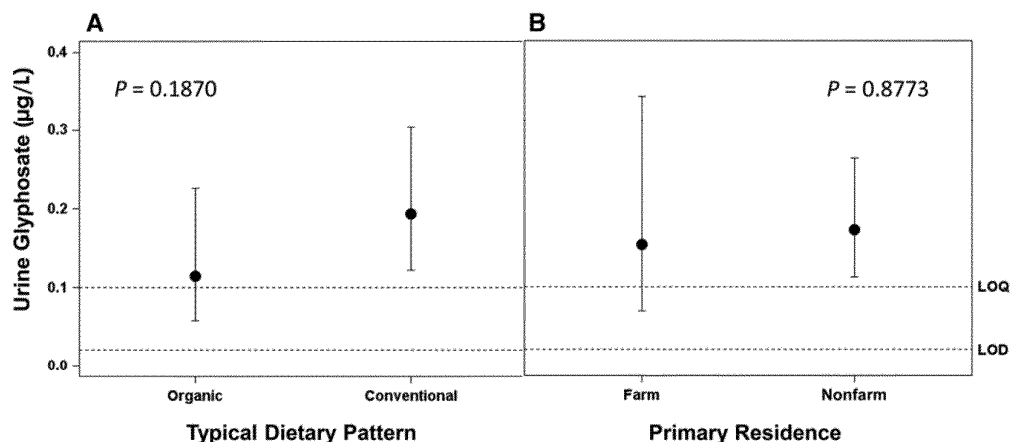


FIGURE 1 Estimated means and 95% CIs for urine glyphosate concentrations of typical self-reported dietary pattern types (A) ($n = 17$ and 23 organic and conventional, respectively) and primary residence types (B) ($n = 10$ and 30 on farm and nonfarm, respectively). Upper and lower reference lines (dashed) represent LOQ and LOD values, respectively. LOD, limit of detection; LOQ, limit of quantification.

healthy adult populations (, 0.15 to 29 and , 0.15 to 1.82 mg/L, respectively) (14, 24–26). To put these values in perspective, it is worth considering the EPA's reference dose (RfD) value for glyphosate. The RfD is an estimate of the quantity of a chemical that a person could be exposed to every day for the rest of his or her life with no appreciable risk of adverse health effects (30). The RfD for glyphosate is $1.75 \text{ g } \$ \text{ kg}^{-2.1} \$ \text{ d}^{-2.1}$; this value is based on a “no-effect” concentration in animals ($175 \text{ mg } \$ \text{ kg}^{-2.1} \$ \text{ d}^{-2.1}$) with a 100-fold safety factor (margin of exposure) (31). The EPA considers AMPA to be of similar or lesser toxicity than glyphosate and determined in 1994 that it should be exempt from regulation regardless of concentrations observed in food or feed (31). Thus, a 75-kg woman (typical weight for our study's participants) could consume as much as 131.25 mg glyphosate/d with no expected negative effects. If 20% of dietary glyphosate is absorbed (i.e., 20% bioavailability) (14) and 100% of absorbed glyphosate is excreted into urine, such an individual would be expected to excrete 26.25 mg/d ($26,250 \text{ mg/d}$) glyphosate in her urine. In the current study, the highest reported

urine glyphosate concentration was 1.93 mg/L . As such, even allowing for a relatively high urine output (3 L/d), the highest glyphosate excretion in our study would be 5.79 mg/d , a value .4500 times lower than that which would be expected if the hypothetical mother described previously had consumed the RfD for glyphosate. The inclusion of AMPA, assuming equivalent toxicity, results in the highest excretion in our study of 2.58 mg/L (7.74 mg/d assuming 3 L urine/d) glyphosate + AMPA, an exposure .3000 times below the RfD; this combined calculation may become important if the EPA reconsiders the safety of AMPA (31).

Applying similar parameters and logic, a 5-kg infant can consume up to 8.5 mg/d (8500 mg/d) glyphosate and be below the RfD of this compound. Assuming a mean milk intake of 0.7 L/d (32–34) and a milk glyphosate concentration of 1 mg/L (the LOD value), then the maximum daily consumption of glyphosate by this hypothetical infant would be 0.7 mg/d —a value , 12,000 times that which is thought to signal any semblance of a health concern (31).

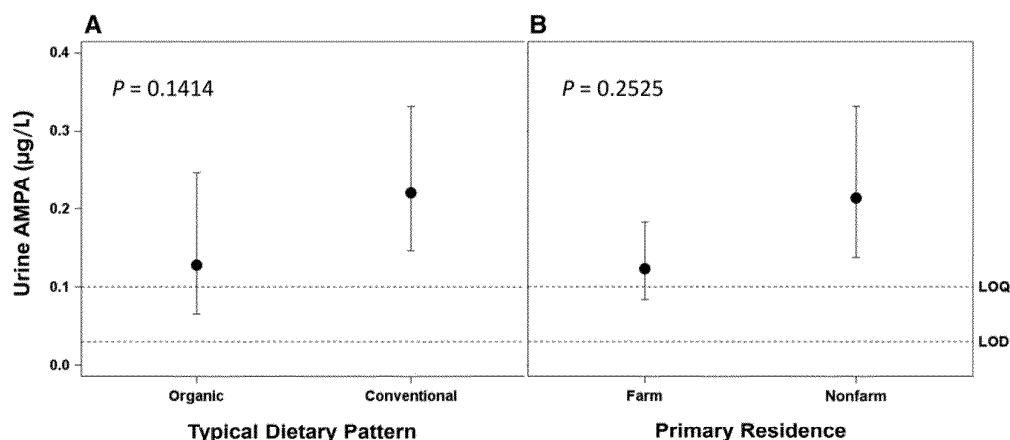


FIGURE 2 Estimated means and 95% CIs for urinary AMPA concentrations as they are related to typical self-reported dietary pattern types (A) ($n = 17$ and 23 organic and conventional, respectively) and primary residence types (B) ($n = 10$ and 30 on farm and nonfarm, respectively). Upper and lower reference lines (dashed) represent LOQ and LOD values, respectively. AMPA, aminomethylphosphonic acid; LOD, limit of detection; LOQ, limit of quantification.

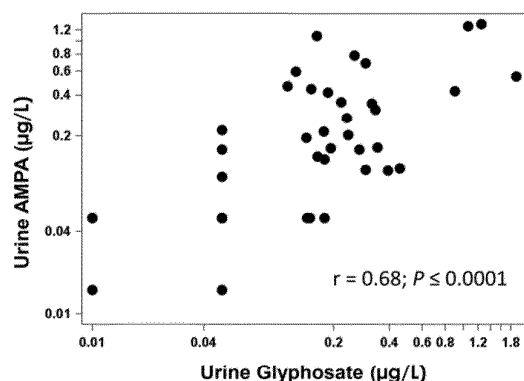


FIGURE 3 Correlation between urine glyphosate and AMPA concentrations. Vertical and horizontal axes represent the natural log scale ($n = 40$; 3 values hidden). AMPA, aminomethylphosphonic acid.

The observed correlation between urine glyphosate and AMPA concentrations is also of interest. Our reanalysis of previously published data from Hoppe et al. (20) suggests correlations (Pearson correlations of 0.40 and 0.68 for raw and log-transformed data, respectively) very similar to our data. Because the strength of association was greater with the use of the log-transformed data, it is likely that this relation is not proportional but rather nonlinear in nature. Whether the AMPA was derived from endogenous metabolism of glyphosate, consumed as a component of the diet, or resulted from exposure to AMPA-containing detergents, however, cannot be determined from our study.

There are some limitations that should be taken into consideration when interpreting the results of this study. First, our subjects were relatively homogeneous in terms of anthropometrics, demographics, and geographical place of residence. Future studies should consider recruiting women of varied educational and ethnic backgrounds living in different regions of the United States. Second, it is noteworthy that the larger international study from which these samples originate was not designed to detect small differences in urine glyphosate and AMPA concentrations based on dietary choices, location of residence (e.g., urban compared with rural), or occupational glyphosate exposure. Nevertheless, we thought it was of topical interest to preliminarily explore those hypotheses given the availability of information. We note, however, that detecting such small-effect sizes at statistically significant concentrations and adequate statistical power would require 4–5 times as many observations than used in this study. Subsequent research on this topic should consider increasing sample sizes to the largest extent possible while targeting enrollment of women who fit the hypotheses of interest, such as rarely or commonly consuming organic food, living on or off farms where glyphosate is used, and/or mixing and applying glyphosate as part of their livelihood. Studies might also consider investigating the possible effect of agricultural season on the outcomes of interest and the potential for breast infection (mastitis) to influence whether glyphosate and AMPA can be detected in a woman's milk. Investigators are also urged to collect urine samples from exclusively breastfed infants to verify the lack of glyphosate and AMPA exposure during this important period of the life cycle and consider collecting complete breast expressions in case glyphosate and AMPA concentrations change during feeding.

Last, studying potential glyphosate and AMPA exposures from other sources (e.g., environmental and supplementary foods) before and after weaning might be of interest. However, it is important to note that glyphosate exposure would need to be much higher than those reported herein for maternal or infant exposures to become a health concern.

The authors' responsibilities were as follows—MKM, MAM, DAG, and JLV: conceptualized and designed the study; MKM and DAG: designed the glyphosate exposure questionnaire; MKM: oversaw sample and data collection; JMC and KAL: collected the samples and administered the questionnaires; PKJ: oversaw the analysis of the samples at Monsanto; WJP and BS: carried out the statistical analysis; and all authors: read and approved the final manuscript. In 2014, MKM and MAM each received a \$10,000 unrestricted research gift from Monsanto; these funds were used to support their research related to human and bovine lactation. These funds were neither needed for nor used to cover the costs associated with the project described in this article, because the milk was already being collected for another project funded by the National Science Foundation (1344288) related to international variation in human milk composition and because additional expenditures associated with the collection of urine samples were negligible. All costs associated with the chemical analysis of milk and urine samples at both Monsanto and Covance were paid for directly by Monsanto. MKM and MAM were once reimbursed for costs associated with economy travel and basic accommodations incurred for a trip they made to St. Louis, Missouri, to discuss study design and assay development with coauthors DAG, PKJ, and JLV at Monsanto. DAG, PKJ, and JLV are employees of Monsanto, which manufactures glyphosate. None of the other authors reported a conflict of interest related to the study.

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